

THE TIME OF OVULATION AND ITS DIURNAL REGULATION IN THE POST-PARTURITIONAL MOUSE¹

MEREDITH N. RUNNER² AND AARON J. LADMAN³

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

TWO FIGURES

Reproductive phenomena in the common laboratory mammals show varying degrees of dependency upon the diurnal cycle. At one extreme is the rabbit which ovulates 9½ to 10½ hours after sexual stimulation; presumably irrespective of the time of day that such stimulation occurs and hence, independent of diurnal influences. Ovulation in the hamster seems to lie at the opposite extreme because this animal adheres to a clock-like schedule and ovulates spontaneously between 12 and 2 A.M. The rat and guinea pig, as reported, seem to be intermediate in so far as ovulation occurs over a wider range of the diurnal cycle but still within the early hours of the morning.

Considerable confusion and inconsistency exists in the literature regarding the time of day of occurrence of that ovulation which closely follows parturition. Boling et al. ('39) expressed surprise to find that in the guinea pig parturitions were evenly distributed throughout the 24 hours of the day while estrus was predominantly nocturnal. Blandau and Soderwall ('41) found that in the rat "the time of day the litter is

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² This work was done while the senior author was a Finney-Howell Research Fellow.

³ Present address: Indiana University School of Medicine, Department of Anatomy.

dropped seems to influence the length of the interval between parturition and heat." Information concerning the time of ovulation in the post-parturitional mouse was reported by Long and Mark ('11) in their classical study on meiosis, fertilization and ovulation. Notwithstanding their conclusion, that the time of ovulation is not rigidly fixed with regard to parturition (p. 361), subsequent reviewers may have unwittingly perpetuated their basic assumption, that events associated with estrus could be timed from parturition, by repeating the time honored statement that the mouse ovulates within 24 hours of parturition. Unfortunately, the data of Long and Mark are not correlated with time of day, but their finding that ovulation occurred from $14\frac{1}{2}$ to $28\frac{1}{2}$ hours after parturition will receive further consideration.

The most thorough study on the time of ovulation in the mouse was conducted at this laboratory on cyclic females (Snell et al., '40). It was found that ovulation usually occurred between 12 midnight and 2 or 3 A.M. Published data on the time of ovulation in mice does not enable one to correlate nocturnal ovulation in cyclic females with the time of ovulation in post-parturitional mice. Calculation shows that animals delivering between 12 noon and 8 P.M., one-third of post-parturitional mice, cannot ovulate at $14\frac{1}{2}$ to $28\frac{1}{2}$ hours post-partum as indicated by Long and Mark and still conform to an ovulation peak close to 1 A.M. as shown by Snell et al. Consequently, one is led to inquire whether post-parturitional animals behave differently from cyclic animals or whether one or both studies on ovulation in the mouse provide data that are too incomplete to explain what actually takes place.

Since a knowledge of the time at which ovulation occurs in mammals is of importance to the endocrinologist, embryologist and student of animal reproduction, additional observations were made in order to demonstrate more clearly the time of post-parturitional ovulation in the mouse. The data presented supplement preliminary observations previously reported (Runner, '47).

MATERIAL AND METHODS

Data were obtained from January to August inclusive from Swiss mice kept in a well ventilated dark room for mice. Light switches, controlled by an electric clock, regulated the only source of illumination and provided uniform alternation of light and dark periods, thereby obviating seasonal influence. Lighting was adjusted to approximate the length of light (18 hours) and darkness (6 hours) occurring on June 21. The dark period came at a time (9 A.M. to 3 P.M.) which would enable convenient and intensive study of nocturnal activities, and which would enable conversion to June solar time simply by changing A.M. to P.M. and P.M. to A.M. The artificial diurnal cycle will be designated in terms of hours 1 to 24 with hour 24 in the middle of the dark period. The light period in the artificial cycle extended from hour 3 to hour 21 and the dark period from hour 21 to hour 3.

Experimental mice were brought into artificial diurnal cycles at 3 to 4 weeks of age. It had been shown previously that two weeks suffice to condition mature mice to reversed light-dark alterations (Snell, Hummel and Abelman, '44) and that rats respond similarly to experimental light alternations whether started at birth or at 21 days (Fiske, '41).

Pregnant mice were removed from the breeding pens, isolated and thereafter observed 6 times per day, every 4 hours at hours 1, 5, 9, 13, 17 and 21. Observations at these hours provided 6 4-hour delivery groups during each diurnal cycle which had average hours of delivery at 3, 7, 11, 15, 19 and 23 (± 2 hours). The process of parturition, requiring about $\frac{3}{4}$ hour (Merton, '38), was sufficiently rapid so that most deliveries were completed within the 4-hour interval. A few animals (20%) were in the process of delivery at the time of one observation. Such animals were classified as belonging to the period during which the majority of the litter was delivered. Pregnant and post-parturitional mice were kept apart from males and the newborn mice were left with the mother until she was prepared for anesthesia and observed for tubal ova. An incision in the lateral body wall enabled observations of ovary

and oviduct for follicles and ova respectively. Whenever tubal ova were present they could be seen and counted through the thin-walled ampulla of the oviduct. If ova were not seen in the first side opened, the opposite side was examined. Following observations the appropriate sutures were made, the animal returned to the colony and she was observed again after the birth of her second and third litters.

We wish to acknowledge with gratitude the assistance of Mr. John Lemay in making the continual observations on pregnant mice.

OBSERVATIONS

Examination for presence of tubal ova was made just once after each of 330 parturitions.⁴ Mice were observed at each hour between 8 and 23 hours post-partum. When the work had progressed far enough, more intelligent planning of observations was possible. A series of animals could be examined at consecutive hours beginning before an appreciable number of animals possessed tubal ova and continuing until several hours were encountered in which most animals had ovulated (see footnote p. 354).

One might expect, *a priori*, as did Long and Mark ('11) and Kirkham and Burr ('13) that ovulation would occur at a fixed interval after parturition. A comparable situation is known for the rabbit where ovulation follows sexual stimulation. One might presume on the other hand that ovulation would be rigidly affixed to a specific portion of the light-dark cycle. Such has been demonstrated for cyclic females in the rat (Young, Boling and Blandau, '41), guinea pig (Young, '37), mouse (Snell et al., '40) and most strikingly in the hamster (Ward, '46). Since either of the above possibilities could oc-

⁴ Observations on the diurnal distribution of deliveries for 492 animals kept under continual observation have substantiated results reported by Long and Mark ('11, 147 deliveries) and Merton ('38, 162 deliveries) in so far as frequent deliveries were found in each sixth of the 24 hours. In our material, no 4-hour delivery period included more than 20% or less than 12% of the total number of observations.

cur in the post-parturitional mouse, the data were tested to see which, if either, was applicable to the observations at hand.

If the mice used for these experiments had ovulated at a fixed time in the light-dark cycle, the frequency with which ova were observed should have been zero before occurrence of ovulation and 100% or close thereto after that time at which ovulation occurred. Data from 270 mice delivering in all parts of the artificial diurnal cycle were observed from 4 hours before to 3 hours after the dark period and within 20 hours of parturition. These data (table 1) show that from 37 to 61% of the mice had tubal ova at each pair of hours observed. A chi

TABLE 1

Frequency of occurrence of tubal ova at given times in the light dark cycle

	HOURS IN THE ARTIFICIAL DIURNAL CYCLE AT WHICH OBSERVATIONS WERE MADE:							TOTAL
	17 and 18	19 and 20	21 and 22	23 and 24	1 and 2	3 and 4	5 and 6	
	Light		Dark			Light		
Number of mice observed	34	43	55	47	31	29	31	270
Number with tubal ova	16	16	29	22	19	15	13	130
Per cent with tubal ova	47.1%	37.2%	52.7%	46.8%	61.3%	51.7%	41.9%	48.1%

square test showed that the pooled observations at these specific times did not differ from the average. This precluded a possibility that animals, as a whole, had significantly different frequencies of tubal ova within this 14-hour period. A modal time for nocturnal ovulation was not demonstrated.

Since the mice examined did not ovulate in a specific part of the artificial nighttime, the alternate possibility was investigated, viz., that ovulation for all animals might have occurred at a fixed and similar interval post-partum. This possibility was tested by comparing (table 2A) the frequency with which ova were seen at 13, 14 and 15 hours post-partum in animals

delivering in different sixths of the light-dark cycle, i.e., at hours 3, 7, 11, 15, 19 and 23 ± 2 hours. If all animals had delivered at a fixed and similar interval, the frequency of appearance of tubal ova at 13, 14 and 15 hours post-partum should have been similar for each of the 6 delivery groups.

Inspection of table 2A shows the frequency of tubal ova for the 6 groups at similar hours post-partum. It is seen that the groups had from 13 to 75% of the animals with tubal ova. A chi square test for homogeneity demonstrated that certain of these groups differed significantly from the average. Hence, some factor or factors other than interval post-partum must have influenced the time of ovulation. The mice observed therefore, cannot be regarded as having ovulated at a consistent interval post-partum.

The data established that collectively, the mice neither had a time at which there was a mode for ovulation in the artificial light-dark cycle (table 1) nor did they ovulate at a fixed interval post-partum (table 2A). It is indicated that either there was no regularity in the time of occurrence of the post-parturitional ovulation or, if a consistent pattern for this ovulation did exist, it was influenced by the time of day at which delivery occurred.

A positive relationship between parturition and ovulation was suggested in table 2A by the progressive increase in the per cent of tubal ova. Those animals which delivered at the end of the light period had ova more frequently, and presumably ovulated at shorter intervals post-partum, than animals delivering during the dark period. The former had 61.9 and 75.0% with tubal ova and the latter had ova in 18.8 and 13.0% of the cases observed. The progressive increase in frequency of ova indicated that the times for observations on the animals delivering in different sixths of the day, if systematically adjusted, might establish a positive relationship between parturition and ovulation.

The frequency of occurrence of tubal ova at each of the 6 average hours of delivery was compared when the intervals

TABLE 2
Tubal ova from animals delivering in different sixths of the diurnal cycle

2A FREQUENCY OF OCCURRENCE OF TUBAL OVA AT 13, 14 AND 15 HOURS POST-PARTUM ¹				2B FREQUENCY OF OCCURRENCE OF TUBAL OVA AT SUCCESSIVELY SHORTER INTERVALS OR ADJUSTED INTERVALS POST-PARTUM ²			
Average hour of delivery	Number of mice observed	Number and per cent with tubal ova		Time of observation, hours post partum	Number of mice	Number and per cent with tubal ova	
		no.	%			no.	%
23 3 { Dark	16	3	18.8	17, 18 and 19 ¹	25	17	68.0
	23	3	13.0	16, 17 and 18	23	11	47.8
7 11 15 19 { Light	25	8	32.0	15, 16 and 17	29	15	51.7
	23	9	39.1	14, 15 and 16	21	14	66.7
	21	13	61.9	13, 14 and 15	21	13	61.9
	20	15	75.0	12, 13 and 14	19	13	68.4
Total	128	51	39.8	Total	138	83	60.1

$$\chi^2 = 3.9, P > .50$$

¹ For corresponding times of day see table 4.

² Adjusted interval post-partum is defined as a one-hour reduction in the interval between parturition and delivery for each succeeding group after hour 23.

post-partum were decreased one hour for each succeeding delivery period (table 2B). Observations for three successive hours were grouped in order to provide larger samples for comparison. This adjustment gave relatively consistent frequencies of tubal ova, 47 to 68% for animals delivering in each sixth of the diurnal cycle. Therefore, animals delivering in different sixths of the diurnal cycle, and otherwise having different frequencies of tubal ova, were made not to differ significantly. The one-hour correction presumably compensated for differences in the 6 groups. The resulting uniform fre-

FREQUENCY OF OCCURRENCE OF TUBAL OVA AT ADJUSTED HOURLY INTERVALS POST-PARTUM

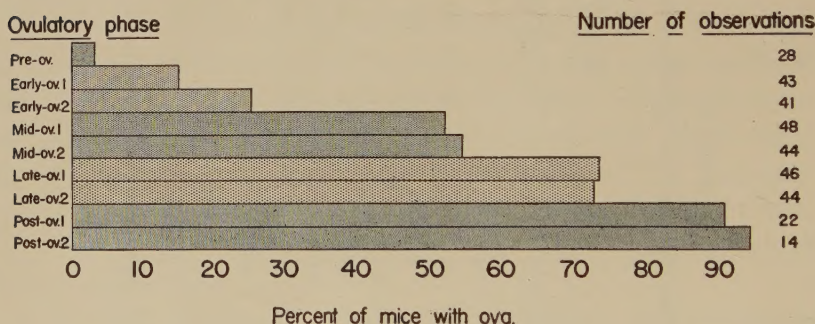


Figure 1

quencies of occurrence of tubal ova did not exclude the possibility that there may have been a positive relation between the time of parturition and the time of ovulation.

A second step was attempted in order to correct for differences in animals delivering in different sixths of the diurnal cycle and thereby, to show a positive correlation between parturition and ovulation. This was accomplished by combining the observations on all 330 mice on the basis of adjusted intervals post-partum. It is apparent that an abrupt increase in the frequency of occurrence of tubal ova for the colony as a whole would suffice to indicate a positive relation between

parturition and ovulation. Application of the one-hour correction to each observation established 5 ovulatory phases. These phases of ovulation were arbitrarily assigned nomenclature in keeping with the observed frequency of occurrence of tubal ova. The ovulatory phases are given in table 3 and illustrated in figure 1. Table 3 gives the results of observations for ova made at two consecutive hours at adjusted intervals post-partum and figure 1 shows the frequency of occurrence of ova at each successive hour at adjusted intervals post-partum.

The tabulation, showing the frequency with which ova were found during the sequence of ovulatory phases (table 3),

TABLE 3
*Frequency of occurrence of tubal ova when comparable ovulatory
phases were combined*

OVULATORY PHASE ¹	NUMBER OF MICE OBSERVED	NUMBER AND PER CENT WITH TUBAL OVA	
		no.	%
Pre-ovulation	28	1	3.6
Early ovulation	84	17	20.2
Mid-ovulation	92	49	53.3
Late ovulation	90	65	72.2
Post-ovulation	36	33	91.6
	$\chi^2 = 97.1$	$p < .01$	

¹ Each ovulatory phase includes the observations made at two consecutive hours at adjusted intervals post-partum and was assigned a name which was in keeping with the frequency of occurrence of tubal ova.

demonstrated a progressive increase in the frequency of appearance of tubal ova from 3.6% at the pre-ovulatory phase to 91.6% at the post-ovulatory phase ($\chi^2 = 97.1$, $P < .01$). Figure 1 illustrates the per cent of mice having tubal ova at each ovulatory phase. The data are given in somewhat more detail in so far as the results are given for each successive hour beginning with the pre-ovulatory and ending with the post-ovulatory phase. Since there was found to be an abrupt increase in the frequency of occurrence of tubal ova between the pre-ovulatory and post-ovulatory phases, a positive correlation between parturition and ovulation was demonstrated. Furthermore, since 3.6% of the animals had ova at the pre-

ovulatory phase and 91.6% had ova at the post-ovulatory phase, the difference between the two, 88%, indicated that most of the animals had ovulated during the intervening 6 hours (dividing into early, mid- and late ovulatory phases).

Having shown that the frequencies of occurrence of tubal ova in post-parturitional mice were similar neither at given times of day (table 1) nor at comparable intervals post-partum (table 2A), but that frequencies were similar after systematically adjusting the intervals post-partum, the results of all observations made for the study are pooled and presented in detail in table 4. The headings for the subdivisions of table 4 show the relation of the 6 parturitional groups to the artificial light-dark cycle and give the average hours of parturition for each of the 6 groups. Hours in the artificial cycle were designated from 1 to 24 where hour 24 was taken as the middle of the dark period. Thus, the 18-hour light portion of the cycle lasted from hour 3 to hour 21 and the 6-hour dark period lasted from hour 21 to hour 3.

Results of observations for tubal ova are arranged vertically in table 4A in 6 columns which correspond with deliveries in each sixth of the diurnal cycle. Horizontally the table presents findings at successive hourly observations at similar phases of ovulation. Having systematically adjusted the intervals post-partum in order to arrive at similar phases of ovulation for all parturitional groups the relation between both times of observations and intervals post-partum are given in tables 4B and 4C respectively. The vertical columns, then, in table 4A show the ratio of animals examined to the animals with tubal ova, those in 4B the corresponding hour post-partum at which observations were made and in 4C the actual time of day at which each successive hourly observation was made.

Table 4A, shows the hourly increase of tubal ova from pre-to post-ovulatory phases for each group of animals delivering in different sixths of the diurnal cycle. The ratio of animals with tubal ova at comparable phases of ovulation (adjusted intervals post-partum) demonstrates that animals delivering in different parts of the artificial diurnal cycle agree rather

TABLE 4

Relation between intervals post-partum, time of day and frequency of occurrence of tubal ova at each ovulatory phase

Artificial cycle Hour of parturition	4A OBSERVATION FOR TUBAL OVA AT SUCCESSIVE HOURS AT ADJUSTED INTERVALS POST-PARTUM							Total % ova	4B NUMBER OF HOURS POST- PARTUM AT WHICH OBSER- VATIONS WERE MADE							4C ACTUAL TIMES AT WHICH OBSERVATIONS WERE MADE								
	< dark 23	> 3	<	7	light 11	15	19		>	< dark 23	> 3	<	7	light 11	15	19	>	< 3	>	7	light 11	15	19	>
Pre-ovulatory phase																								
Number examined	6	7	7	6	6	6	2	28	3.6	14	13	12	11	10	9		11 AM	3 PM	7 PM	11 PM	3 AM	7 AM	4 PM	
Number with ova	0	0	1	0	0	0	0	1									1 AM	4 AM	7 AM	10 AM	1 PM	4 PM		
Early ovulatory phase (1)									14.0	15	14	13	12	11	10		2 AM	5 AM	8 AM	11 AM	2 PM	5 PM		
Number examined	4	9	7	7	5	11	43																	
Number with ova	3	1	0	1	0	1	6		26.8	16	15	14	13	12	11		3 AM	6 AM	9 AM	12 N	1 PM	6 PM		
Early ovulatory phase (2)																								
Number examined	4	7	8	9	7	6	41																	
Number with ova	4	2	2	1	1	1	11		52.1	17	16	15	14	13	12		4 AM	7 AM	10 AM	1 PM	2 PM	7 PM		
Mid-ovulatory phase (1)																								
Number examined	7	8	10	8	9	6	48																	
Number with ova	4	3	6	4	4	4	25		54.5	18	17	16	15	14	13		5 AM	8 AM	11 AM	2 PM	3 PM	8 PM		
Mid-ovulatory phase (2)																								
Number examined	8	9	9	6	6	6	44																	
Number with ova	6	3	5	4	3	3	24		73.9	19	18	17	16	15	14		6 AM	9 AM	12 N	3 PM	4 PM	9 PM		
Late ovulatory phase (1)																								
Number examined	10	6	10	7	6	7	46																	
Number with ova	7	5	4	6	6	6	34		70.5	20	19	18	17	16	15		7 AM	10 AM	1 PM	4 PM	5 PM	10 PM		
Late ovulatory phase (2)																								
Number examined	6	17	6	2	6	7	44																	
Number with ova	4	8	6	2	5	6	31		90.9	21	20	19	18	17	16		8 AM	11 AM	2 PM	5 PM	6 PM	11 PM		
Post-ovulatory phase (1)																								
Number examined	6	6	6	2	1	1	22																	
Number with ova	5	6	5	2	1	1	20		92.9	22	21	20	19	18	17		9 AM	12 N	3 PM	6 PM	7 PM	12 N		
Post-ovulatory phase (2)																								
Number examined	6	6	2	2	2	2	14																	
Number with ova	6	5	2	2	2	2	13																	

Bold-faced type represents observations during the dark portion of the artificial diurnal cycle.

well with each other. Closer inspection of the table, however, indicates that animals delivering at hour 23 on the average had a relatively high proportion of tubal ova in the early ovulatory phase. These animals deviated from the average by ovulating somewhat earlier than animals delivering in other sixths of the diurnal cycle. More will be said of the tendency for precocious appearance of ova in the 23 hour groups. Nonetheless, the overall picture enables one to conclude that each group of animals ovulated at a characteristic time which, in relation to parturition, was relatively fixed.

Comparison of tables 4A, 4B, and 4C enables a study of the relation between the times of parturition, intervals post-partum and times of ovulation. Table 4B shows the manner in which the intervals post-partum were adjusted, i.e., after deliveries at hour 23, the intervals were one hour shorter for each succeeding parturitional group. This correction, justified by the frequencies of tubal ova observed, demonstrated that the mid-ovulatory phases for all 6 groups occurred between 12 and 18 hours post-partum. As a consequence of this progressive shortening of the intervals post-partum, ovulations were not equally distributed throughout the day. As deliveries became later in the day, the times of ovulations occurred relatively, but not absolutely, earlier by one hour.

Table 4C shows that, in terms of actual times of observation, the characteristic times for mid-ovulation in each of the 6 parturitional groups were between 4 A.M. and 8 P.M. In other words, mid-ovulation for the whole series of observations on animals delivering in all parts of the artificial diurnal cycle, occurred within the dark period (9 A.M. to 3 P.M.) plus or minus 5 hours.⁵ This clumping of the times for ovulation resulted in

⁵ It was found expedient during the latter part of the experiment to estimate the time of the mid-ovulatory phase (dark period 9 A.M. to 3 P.M.) by determining the number of hours after 12 noon that delivery occurred, multiplying this figure by $\frac{3}{4}$ and adding the product to 5 A.M. (e.g., delivery at 4 P.M. would place the mid-ovulatory phase at $4 \times \frac{3}{4} + 5$ A.M. or 8 A.M.). Had the observations been made under conditions where the dark period was 9 P.M. to 3 A.M., presumably the mid-ovulatory phase would be reckoned from the number of hours after midnight multiplied by $\frac{3}{4}$ and added to 5 P.M. (e.g., delivery at 4 P.M. would place the mid-ovulatory phase at $16 \times \frac{3}{4} + 5$ P.M. or 5 A.M.).

a situation where animals delivering between the beginning of the light period, hour 3 or 3 P.M. (table 4C) and almost to the end of the light period, hour 19 or 7 A.M., ovulated close to or within the following dark period. Animals delivering during the early part of the dark period, hour 23 or 11 A.M., showed

RELATIONSHIP BETWEEN THE LIGHT-DARK CYCLE AND POST-PARTURITIONAL OVULATION

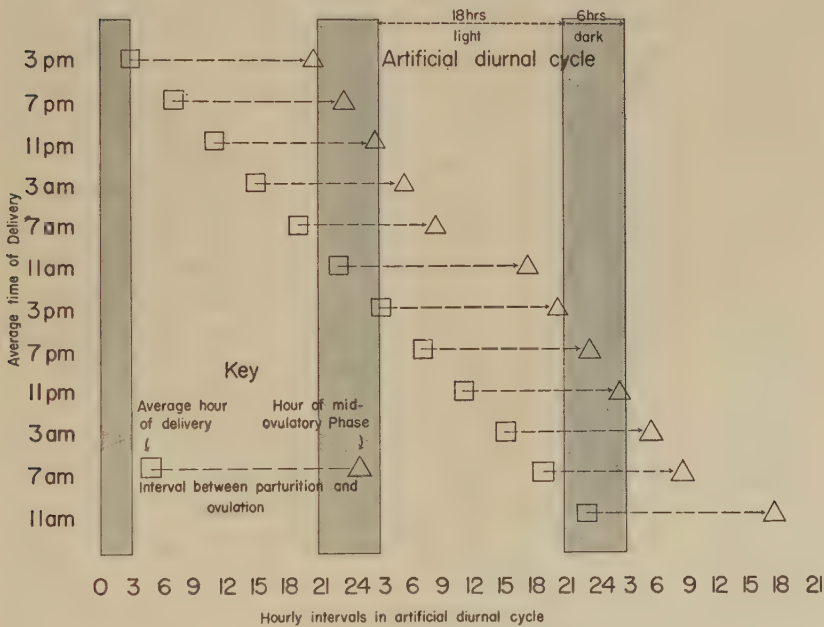


Figure 2

relatively high proportions of ovulations in the early ovulatory phase. These ovulations occurred just after the middle of the light period, hence these animals showed the least tendency to have nocturnal ovulation.

The relation between the light-dark cycle, average times of parturition and characteristic times for mid-ovulation are illustrated in figure 2. Here is shown diagrammatically that the intervals between parturition and ovulation became pro-

gressively shorter after deliveries near the beginning of the dark period, hour 23 or 11 A.M. This progressive shortening of the interval by one hour continued until the animals delivering at hour 19 or 7 A.M. had an interval of 12 to 13 hours. The next succeeding group delivering at hour 23, 4 hours later, had an interval of 17 to 18 hours. This comparison showed an abrupt change in the interval post-partum for ovulation in animals delivering at hours 19 and 23. Such a change is undoubtedly associated with the observations made above that the animals in the group delivering at hour 23 had tubal ova at a somewhat high frequency, hence, they ovulated earlier than would be indicated by observations on the remainder of the animals. Those animals in the hour 23 group may further have been complicated by the fact that their mid-ovulatory phase occurred during the latter half, but well within, the light period. Such a propensity to ovulate during daylight hours is contrary to the situation found in cyclic animals and undoubtedly represents a condition peculiar to certain of the post-parturitional mice. These animals, delivering at hour 23, may have been a transitional group composed of individuals which tended to ovulate slightly behind a schedule indicated by the next succeeding group and ahead of a schedule indicated by the groups delivering prior to hour 23. Such would have been the case if some animals in the group had tended to ovulate nocturnally in one diurnal cycle and some had tended to do so in another cycle.

It may be concluded that factors which were regulating ovulation to occur during the artificial nighttime acted similarly upon those animals delivering in similar portions of the diurnal cycle. Such animals ovulated at relatively fixed intervals post-partum and at characteristic times of the day. Factors determining the times for ovulation in animals delivering in different parts of the diurnal cycle, on the other hand, acted differently upon each group. These animals differed with reference to the intervals post-partum at which ovulation occurred. It would seem that the mechanism by which diurnal factors ordinarily operate to bring about nocturnal ovulation

was disrupted by parturitions in the early part of the dark period so that nocturnal regulation of ovulation was incomplete. Incomplete though it may be for certain groups, we were able to demonstrate that, irrespective of the time of day of parturition, there was an expressed tendency for ovulation to become nocturnal.

DISCUSSION

Some comment seems to be in order regarding the relation of the time of parturition to the time of ovulation since Long and Mark ('11) interpreted their data to show that no correlation existed between parturition and ovulation. Data presented in this report lead to the opposite conclusion. Indeed, the time of the post-parturitional ovulation in the mouse can be predicted within a few hours and this is as accurate as can be done for ovulations in other laboratory mammals, e.g., rabbit, rat, guinea pig, hamster. Conclusions of Long and Mark were based on something more than 22 animals which had eggs at appropriate stages of meiosis. Eggs were found in the ovary, peri-ovarial space, or oviduct and it is unfortunate that the time of day of parturitions and observations was not reported. Examination of the pertinent data (ref. cit., table 4, p. 353) reveals that 16 out of the 22 cases may have come within the mid-ovulatory phases (12 to 18 hours p.p.) as delimited by the present report. Subsequent to the mid-ovulatory phases two of their animals failed to ovulate (at $22\frac{3}{4}$ and $28\frac{1}{2}$ hours p.p.). There is no a priori basis for determining whether these two animals would have ovulated subsequently or whether they had undergone anovulatory estrus. Furthermore, data of the present report do not favor one or the other interpretations, since anovulatory animals may have been encountered in 3 out of 36 animals that did not possess tubal ova during the post-ovulatory phase (16 to 22 hours p.p.).

The above considerations indicate that the animals observed by Long and Mark may have given results parallel to those of the present report in spite of the divergent conclusions. The more complete nature of the latter study, however, has enabled demonstration of a positive and significant correlation between

parturition, ovulation and diurnal cycle. It is to be hoped that traditional statements describing the time of post-parturitional ovulation can be amended to read: the post-parturitional mouse tends to ovulate nocturnally irrespective of the time of day that delivery occurs, and that mice which deliver at similar times of day ovulate at a relatively fixed interval post-partum. This interval, varying between 12 and 18 hours, depends upon the time of day that delivery occurs.

Relationship between the times of parturition, mid-ovulation and the diurnal cycle, illustrated in figure 2, shows that deliveries at the end of the dark period were followed by the longest interval post-partum. This is contrasted with deliveries just before the dark period which were followed by the shortest intervals post-partum. The fact that the shortest period of time between parturition and mid-ovulation was about 12 hours (under conditions where the interval would necessarily have been 8 hours in order that ovulation take place in the dark) suggests that 12 hours was the minimum amount of time post-partum needed for the ovulation inducing reaction to take place. Since within any one parturitional group ovulation times were relatively consistent and predictable, whereas between any two parturitional groups ovulation times were different, the groups of animals must have been affected differently, nonetheless positively, by the ovulation inducing mechanism.

It was found that the ovulation inducing machinery took 5 hours longer to bring about mid-ovulation following deliveries at hour 23 than it did after the deliveries at hour 19. This indicates that following deliveries at times other than hour 19 there was either a delay in getting the ovulation inducing machinery into operation or there was a prolongation of the ovulation inducing process. The above discussion does not imply that parturition is the stimulus for ovulation. The discussion stems from the fact that post-parturitional females within any time group did ovulate at similar times. Parturition may well have been an expression of some event concomitant with initiation of the ovulation inducing machinery.

Any explanations to account for the differences in mice which delivered at hour 19 and those which delivered 4 hours later must be based on information which is not yet available.

It is currently proposed that the pituitary and the ovary interact to produce the events under discussion. More specifically it may be stated that, under the influence of the pituitary, ovarian follicles undergo growth and differentiation. Shortly prior to ovulation the estrogen-progesterone balance initiates the release of a surge of hormone from the pituitary which acts as an ovulatory stimulus. On the basis of these considerations and upon the assumption that the ovulation inducing reaction starts synchronously with parturition the following possibilities need to be examined. Either (a) the ovary at times other than hour 19, e.g., hour 23, was temporarily refractory and took longer to respond to pituitary changes concomitant with parturition or (b) the ovary was equally responsive at any time of day but the pituitary responded by releasing ovulation inducing hormone at different rates at different times of day. One cannot preclude the possibility that there may have been a combination of (a) and (b).

If the pituitary started to release ovulation inducing hormone concomitantly with parturition then, synchronously with parturition at hours 19 and 23, the pituitary started to bring the bloodstream level of gonadotrophin to the pre-ovulatory level but the ovary responded nocturnally. If, on the other hand, the pituitary became active concomitantly with parturition both at hours 19 and 23, but in the latter instance required 5 hours longer to bring the gonadotrophin to the pre-ovulatory level, then the ovary may have responded as promptly as it was able. The former possibility attributes to the ovary a diurnal rhythm in its ability to respond to hormones in the blood stream. The latter possibility attributes to the pituitary a diurnal rhythm in its ability to affect the bloodstream level of gonadotrophin. This latter possibility finds support in the ingenious experiments of Everett and Sawyer ('49) who reported evidence of a 24-hour rhythm in the secretion of an ovulatory hormone.

SUMMARY

Continuous observation of 492 pregnancies under experimentally controlled light-dark alternations showed parturitions to be distributed throughout the 24-hour day. No 4-hour delivery period included more than 20% or less than 12% of the total deliveries observed.

Examinations for the presence or absence of tubal ova were made on 330 post-parturitional mice, one series at each hour between 8 and 23 hours post-partum. This colony of animals was found not to ovulate at a narrowly delimited portion of the light-dark cycle, thereby differing from animals which ovulate during the ordinary estrous cycle.

The frequency of occurrence of tubal ova at 13, 14 and 15 hours post-partum showed the animals delivering in different parts of the diurnal cycle to differ significantly. This observation confirms the findings of Long and Mark that ovulation in a pooled sample is not rigidly fixed with reference to parturition alone. Ovulation frequencies did show a positive correlation with parturition when the intervals between parturition and observation were decreased one hour for each succeeding 4-hour delivery group after beginning of the dark period. This treatment demonstrated natural classifications into a pre-ovulatory phase (3% with tubal ova), early ovulatory phase (20% with tubal ova), mid-ovulatory phase (53% with tubal ova), late ovulatory phase (72% with tubal ova) and post-ovulatory phase (91% with tubal ova). The early, mid- and late ovulatory phases common to all post-parturitional mice covered a period of 6 hours during which 88% of ovulations occurred. The data provided predictable times of ovulation sufficiently accurate for most experimental purposes.

It is concluded that post-parturitional mice tend to ovulate nocturnally irrespective of the time of day that delivery occurs and that mice which deliver at similar times of day ovulate at a characteristic time and at a relatively fixed interval post-partum. This interval, varying between 12 and 18 hours, depends upon the time of day that delivery occurs. Possible

mechanisms for the nocturnal regulation of ovulation are discussed.

LITERATURE CITED

- BLANDAU, R. J., AND A. L. SODERWALL 1941 Post-parturitional heat and the time of ovulation in the albino rat. Data on parturition. *Anat. Rec.*, 81: 419-431.
- BOLING, J. L., R. J. BLANDAU, J. W. WILSON AND W. C. YOUNG 1939 Post-parturitional heat responses of newborn and adult guinea pigs. Data on parturition. *Proc. Soc. Exp. Biol. and Med.*, 42: 128-132.
- EVERETT, J. W., AND C. H. SAWYER 1949 The blocking effect of nembutal on the ovulatory discharge of gonadotropin in the cyclic rat. *Proc. Soc. Exp. Biol. and Med.*, 71: 696-698.
- FISKE, V. 1941 Effects of light on sexual maturation, estrous cycle and the anterior pituitary of the rat. *Endocr.*, 29: 187-196.
- KIRKHAM, W. B., AND H. S. BURE 1913 The breeding habits, maturation of the eggs and ovulation of the albino rat. *Am. J. Anat.*, 15: 291-318.
- LONG, J. A., AND E. L. MARK 1911 The maturation of the eggs of the mouse. Carnegie Institution, Wash. Publ. no. 142, 1-72.
- MERTON, H. 1938 Studies on reproduction in the albino mouse. I. The period of gestation and the time of parturition. *Proc. Roy. Soc. Edinb.*, 58: 80-97.
- RUNNER, M. N. 1947 Attempts at in vitro semination of mouse eggs. *Anat. Rec.*, 99: 564-565.
- SNELL, G. D., E. FEKETE, K. P. HUMMEL AND L. W. LAW 1940 The relation of mating, ovulation and the estrous smear in the house mouse to time of day. *Anat. Rec.*, 76: 39-54.
- SNELL, G. D., K. P. HUMMEL AND W. H. ABELMANN 1944 A technique for the artificial insemination of mice. *Anat. Rec.*, 90: 243-253.
- YOUNG, W. C. 1937 The vaginal smear picture, sexual receptivity and the time of ovulation in the guinea pig. *Anat. Rec.*, 67: 305-325.
- YOUNG, W. C., J. L. BOLING AND R. J. BLANDAU 1941 The vaginal smear picture, sexual receptivity and time of ovulation in the albino rat. *Anat. Rec.*, 80: 37-45.
- WARD, M. C. 1946 A study of the estrous cycle and the breeding of the golden hamster, *Cricetus auratus*. *Anat. Rec.*, 94: 139-162.

MORPHOGENESIS OF THE VITELLINE AND ALLANTOIC PLACENTAE OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)¹

FRANK W. ADAMS AND HOWARD H. HILLEMANN

Department of Zoology, Oregon State College, Corvallis

TWELVE FIGURES

INTRODUCTION

Of the many papers on the development of rodent placentae, only those of Graves ('45), Venable ('46) and Ward ('48) are concerned with hamsters. Venable was interested in pre-implantation stages; Graves studied both embryo and membranes through the first 9 days of gestation; and Ward considered implantation and associated endometrial changes into the 5th day of gestation.

This paper deals with the morphogenesis of hamster placentae (vitelline and allantoic) from the 6th day of gestation to term (day 16).

MATERIALS AND METHODS

The placentae of 21 pregnant hamsters representing days 6 through 16 of gestation were studied. Fetal age was determined by the lapse of time from observed coitus to sacrifice. All loculi were fixed in Bouin's and to accelerate fixation of the larger loculi, they were injected also.

The paraffin-embedded loculi were serially sectioned at 6-10 μ and stained with hematoxylin and eosin. For proper

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paraffin infiltration of the larger loculi, three days at low temperature (56°C.) were required. At least 5 paraffin changes were made during this period.

OBSERVATIONS

Sixth day of gestation

Embryo. The blastocyst is composed of a single layer of trophoblast cells and an inner cell mass which nearly fills the blastocyst cavity. The trophoblast is slightly flattened at the antimesometrial pole (fig. 1).

Uterus. The blastocyst is enclosed in an implantation chamber (cup) of the uterus. Points of contact between the blastocyst and uterus are characterized by complete erosion of the uterine epithelium except for a few disintegrating epithelial cells adjacent to the mesometrial surface of the blastocyst. Elsewhere the uterine epithelium shows no degeneration. The implantation reaction manifests itself in the endometrium as a thickening in the decidua, the portion adjacent to the embryo (primary decidua) being more compact than that adjacent to the muscularis (secondary decidua). In the decidua near the embryonic area are maternal blood sinuses lined with an intact endothelium. The peripheral portion of the endometrium consists of many vacuolated cells, the contents of which were dissolved out during fixation and dehydration. The uterine glands have been pushed toward the muscularis, except for a few which remain near the lumen on the mesometrial side.

Seventh day of gestation

Embryo. The embryonic trophoblast approaches from either side to form an amniochorionic canal connecting two cavities, one being the true amniotic cavity antimesometrially, and the other the false amniotic cavity (chorionic cyst, ectoplacental cavity, epamniotic cavity) mesometrially. A layer of entoderm is reflected over the outside and extends in a "U" shape around the embryo. A double layer of mesoderm, containing the incipient extraembryonic coelom, lies between

the ectoderm and the entoderm. The trophoblast nuclei, having left a cytoplasmic residue organized into Reichert's membrane, invaded the stroma where they transformed into giant cell nuclei.

A dome shaped ectoplacental cone (Träger) consisting of a dense mass of deeply staining cells has been proliferated from the mesometrial lamina (ectoderm) of the amniotic cavity.

Uterus. The epithelium of the implantation chamber is entirely eroded leaving a residue of cellular debris, and a thin bridge of stroma represents the closure between the implantation chamber and the uterine lumen proper. The epithelium lining the uterine lumen at the level of the locus shows extensive erosion with a few degenerating epithelial cells remaining along the mesometrial surface. The entire mesometrial stroma as well as the antimesometrial stroma near the muscularis, is compact in appearance while the remaining antimesometrial stroma is highly vacuolated.

The endothelial lined blood sinuses have multiplied, the greater number being in the mesometrial half of the endometrium.

In comparison with their location on the previous day, the glands in the antimesometrial portion of the endometrium have been pushed farther toward the muscularis.

Seventh day of gestation (late)

Embryo. The true and false amniotic cavities are now separate. Entodermal and ectodermal layers of the embryo are well differentiated and a mesodermal layer is visible in the false amniotic cavity.

The placental cone has thickened, and located within its folds are lacunae of maternal blood which have no endothelium but are bounded by placental cone cells only.

Between Reichert's membrane and the stroma are many accumulations of maternal red blood cells.

Uterus. The few remaining glands are crowded against the muscularis. The uterine lumen has been greatly reduced and the lining epithelium entirely eroded.

Eight day of gestation

Embryo. The amnion has become a large attenuated sac of ectoderm covered by somatic mesoderm. The cells of this somatic mesoderm and of the splanchnic mesoderm of the yolk sac are increasingly larger toward the embryo. Between these two mesodermal layers and continuous with each other are the cavities of the intraembryonic and extraembryonic coeloms. Somatic mesoderm completely lines the peripheral wall of the large exocoelom and is forced as a thin membrane against the antimesometrial lamina (ectoderm) of the false amniotic cavity.

The entoderm proximal to the embryo appears as an attenuated strand, but in the area of the exocoelom its cells are cuboidal and lie adjacent to newly formed blood islands. The entoderm follows the peripheral ectodermal wall of the false amniotic cavity until it meets the stroma of the endometrium where it deflects sharply and proceeds in a reverse direction as a very thin line against Reichert's membrane (fig. 3). The small nuclei of the parietal (outer, non-persistent, ephemeral) entoderm extend around the yolk sac cavity. Antimesometrially, these nuclei are larger and project in endothelial fashion into the yolk sac cavity.

Beyond the parietal yolk sac is Reichert's membrane with an occasional nucleus like those of typical giant cells lying beside it. Reichert's membrane is apposed to the outer yolk sac layer, except for occasional spaces containing maternal red blood cells in various stages of disintegration.

Allantois. The allantois has progressed across the extraembryonic coelom approximately half way. It is composed of a loose network of splanchnic mesodermal cells taking origin from the posterior end of the embryo.

The central area of the antimesometrial lamina (ectoderm) of the false amniotic cavity along with the adherent somatic

mesoderm is pressed against the central area of the mesometrial lamina (ectoderm), converting the false amniotic cavity proper into a circular canal.

Uterus. There is no evidence of a uterine epithelium. The border of the uterine lumen is formed by maternal blood lacunae apposed to the aligned parietal yolk sac cells. Giant cells, present in the stroma, are more numerous on the mesometrial side of the uterine lumen.

The placental cone encloses many maternal lacunae which lack an endothelium. Much of this blood, in direct contact with placental cone tissue, appears to be hemolyzed.

Ninth day of gestation

Membranes. The amnion is highly attenuated with the nuclei of the ectoderm alternating with the nuclei of the outer somatic mesoderm.

The visceral (inner, permanent) yolk sac consists of a simple cuboidal epithelium overlying the splanchnic mesoderm. In this splanchnic mesoderm are fetal blood vessels containing nucleated red blood cells (fig. 5). The parietal entoderm is nonvascular.

The area of the stratum compactum invaded by the giant cells has lost its cellular organization and is composed of an amorphous material with clear vacuoles. Previously the giant cells had been observed to migrate in all directions from the implantation chamber, but at this stage a more advanced migration is found mesometrial to the embryo (fig. 4).

The placental cone, previously dome-shaped, becomes definitely conical with the margins of the apex in section forming an acute angle (fig. 2). Enclosed in the interstices are maternal red blood cells in various stages of dissolution along with trophoblastic endovascular plasmodial cells (Bridgeman, '48). Adjacent to the cone, the migrating giant cells have isolated a section of decidua with a vacuolated appearance (fig. 4). This conversion of maternal tissue may be the result of the action of the giant cells, since it lies behind their line of advance.

Allantois. The allantois had traversed the extraembryonic coelom bringing the endothelium of the allantoic blood vessels in contact with the combined somatic mesoderm and antimesometrial lamina (ectoderm) of the false amniotic cavity. A smooth splanchnic mesodermal membrane derived from the allantois extends across the allantoidean placental area (fig. 4). These endothelial lined allantoic blood vessels filled with nucleated red blood cells extend as finger-like projections into the antimesometrial side of the placental cone. Thus, separating the maternal red blood cells from the fetal blood are the following membranes: placental cone ectoderm, somatic mesoderm, and allantoic endothelium. This combination forms a thick villous hemochorial placenta (fig. 6).

Uterus. A cross section of a maternal blood vessel in the mesometrium reveals a very hypertrophied endothelium with nuclei bulging into the lumen. Inside this particular blood vessel and occupying almost all of the cross sectional area is found a trophoblastic endovascular plasmodial cell previously noted, and pictured in the rat by Bridgeman ('48). In one maternal artery of the mesometrium was seen an endovascular plasmodial cell along with many laked red blood cells surrounding scattered vacuoles. It may be that the plasmodial cell is responsible for this dissolution. This cell is much larger than the giant cell, has a large blue staining nucleus and a lavender mass of cytoplasm.

Antimesometrially, the epithelial lined uterine lumen is being re-established, separating the decidua capsularis from the uterine wall.

Tenth day of gestation

Membranes. The visceral yolk sac epithelium of cuboidal cells is thrown into folds and villi which project into the yolk sac cavity and are vascularized by endothelial lined vessels containing nucleated red blood cells derived from a well developed layer of splanchnic mesoderm.

Immediately outside the parietal yolk sac are many extensive blood spaces enclosed by a network of cytoplasmic

processes projecting from giant cells. These spaces are filled with maternal blood.

Many maternal blood cells in various stages of disintegration, as well as scattered nuclei of giant cells, are seen between Reichert's membrane and the parietal yolk sac. Blood bathing the yolk sac in this manner is suggestive of a fetal nutrition through a vitelline placenta subsidiary to that of the chorioallantois.

Allantoidean placenta. The endothelium of the allantois has grown through the chorion or serosa (somatic mesoderm + antimesometrial lamina + mesometrial lamina) to contact the placental cone cells. The placenta is now hemochorial but has no somatic mesodermal layer in this barrier between fetal and maternal blood. Some endothelial blood vessels from the allantois pass through the chorioallantois and form larger vessels between the placental cone and the uterine stroma.

The giant cells have penetrated the basalis, isolating an area which in turn is broken up by large maternal blood spaces. The cells of this isolated basalis are reduced in size. Surrounding the giant cells are remnants of disintegrating stroma.

Uterus. The uterine stroma of the basalis is highly vascularized, containing many large endothelial lined blood vessels as well as lacunae lacking an endothelium.

Antimesometrially, the decidua basalis is divided into a medial portion bounding the decidua capsularis and a lateral part remaining attached to the muscularis. The space between represents the re-established uterine lumen and is lined on either side with a new epithelium (fig. 7).

Eleventh day of gestation

Membranes. The visceral yolk sac is increasingly vascularized.

Allantoidean placenta. The chorioallantoic placenta remains hemochorial, consists of cone ectoderm and allantoic endothelium, and is labyrinthian in its arrangement.

The placental cone itself is broken up by maternal lacunae lined by stromal cells with clear vacuoles representing the position of cellular inclusions removed by fixation and clearing.

The giant cell nuclei have grown further and attained a diameter about 20 times that of the red blood cells.

Uterus. Antimesometrially, the stroma has been compressed and stretched until it is no greater in thickness than the combined muscularis and serosa. The muscularis is also narrowed. In this region, the uterine lumen is complete and lined with epithelium.

Twelfth day of gestation

Membranes. Antimesometrially from the amnion, there are 6 structures: (1) the convoluted and highly vascularized villous visceral yolk sac composed of simple squamous splanchnic mesoderm medially and a convoluted villous entoderm laterally; (2) the yolk sac cavity limited by the parietal yolk sac membrane composed of simple epithelium with spherical nuclei bulging into the yolk sac lumen; (3) Reichert's membrane, appearing as a definite non-cellular layer apposed to the parietal yolk sac; (4) the remaining stroma; (5) the muscularis composed of circular and longitudinal smooth muscle; (6) the serosa, composed of simple squamous epithelium.

Between Reichert's membrane and the stroma are lacunae of maternal blood. The stroma is bounded peripherally by simple cuboidal epithelium of the re-established uterine lumen. This lumen has an outer columnar epithelium apposed against a layer of basalis, which will remain after birth.

Mesometrially, the visceral and parietal yolk sacs and Reichert's membrane have not undergone any changes from the previous day.

Allantoidean placenta. Capillaries from allantoic blood vessels projecting mesometrially, have thoroughly broken up the remnants of the placental cone leaving only scattered ectodermal cells. The allantoic capillaries course in irregular lines in a mesometrial direction and are paralleled by sim-

ilarly arranged slender blood lacunae containing maternal blood. Only the endothelium of the fetal capillaries is interspersed between fetal and maternal blood streams. Thus, a hemoendothelial placenta, labyrinthian in arrangement, has been established (fig. 8).

Mesometrially to the region of apposition of fetal and maternal circulations, there are irregular islands of the original placental cone cells. These islands have been isolated by maternal blood lacunae, which also break up the basalis.

Integral chorioallantoic and yolk sac placenta. Visceral yolk sac blood vessels project through the parietal yolk sac and Reichert's membrane into the apposed allantoidean placenta. For a short distance the epithelium of the two yolks sacs together with Reichert's membrane accompany the blood vessels, but farther along the barrier between maternal and fetal blood is represented by only visceral yolk sac vascular endothelium.

Twelfth and one-half day of gestation

Membranes. Mesometrially the convoluted visceral yolk sac membrane has become more villous with secondary fingers. It is highly vascular in the area of the allantoidean placenta.

Allantoidean placenta. The allantoidean placenta remains hemoendothelial. The placental cone is thoroughly broken up with its ectodermal cells spread along a line called the junctional zone.

Thirteenth day of gestation

Allantoidean placenta. The allantoidean placenta has extended its boundary centrifugally in such a manner that its outer circular margin overlaps the yolk sac. Thus, arranged from the embryo toward the mesometrium are: the amnion; the convoluted and vascular visceral yolk sac and yolk sac cavity; the parietal yolk sac; Reichert's membrane; and the allantoidean placenta proper comprising maternal blood

spaces, isolated cone cells and allantoic endothelial vessels containing fetal red blood cells.

Large maternal blood lacunae exist between the allantoidean placenta and Reichert's membrane (fig. 9), and visceral yolk sac blood vessels penetrate the allantoidean placenta as on day 12.

Fourteenth and one-half day of gestation

Membranes. The amnion is followed peripherally by the visceral yolk sac composed of an inner layer of a simple squamous splanchnic mesoderm and an outer layer of cuboidal entoderm. Reichert's membrane, the yolk sac and the vitelline cavity have disappeared in a limited area from the antimesometrial side.

Maternal blood lacunae lie between the visceral yolk sac and the re-established uterine lumen. It should be noted that the medial uterine epithelium which was present on the 13th day has disappeared. Thus, the maternal blood lies between the visceral yolk sac layer and the outer epithelium of the new lumen (complete inversion of germ layers). See figure 11.

Outside the re-established uterine epithelium is a thin layer of basilar stroma. Maternal blood vessels occur directly against the epithelium and also within the stroma. Outside the basilar stroma are the circular and longitudinal smooth muscles covered by the serosa.

Proceeding toward the mesometrial portion of the uterus, the visceral yolk sac is thrown into progressively more complicated and longer villi, all of which are vascularized. The parietal yolk sac, as an attenuated layer with nuclei bulging into the yolk sac lumen, extends only as far as the hypertrophied basalis adjacent to the allantoidean placenta. Reichert's membrane lies against the parietal yolk sac and extends from the allantoidean placenta, disappearing before it reaches the antimesometrial pole.

Allantoidean placenta. The allantoidean placenta, which is more extensive than before, remains hemoendothelial in structure. Some placental cone ectoderm cells are isolated

while others form a definite boundary or junctional zone (Bridgeman, '48) between the allantoidean placenta and the decidua basalis. Immediately mesometrial to the junctional zone, the basalis is composed of stromal cells heavily permeated by maternal blood lacunae having no endothelial lining. Numerous giant cells are present, both in the basalis and among the cells of the junctional zone.

Passing from the allantois through the allantoidean placenta to the junctional zone are a series of allantoic vessels composed solely of an endothelium separated from the allantoidean placenta proper by a loose scattered cell network (fig. 10).

Very few giant cells are found free within the allantoidean placenta, but there are a number of dark staining and closely packed circular islands of cells among which an occasional giant cell may be found. No explanation is offered for these islands other than that suggested by their resemblance to the original placental cone cells.

Uterus. In the mesometrial region, the uterine epithelium of the re-established lumen is thrown into numerous villi which project into the lumen itself. This epithelium is not present on the medial (toward the embryo) side of this lumen.

Fifteenth day of gestation

Allantoidean placenta. The allantoidean placenta presents its maximum development (fig. 12). The area of exchange is more vascular than before and is composed of allantoic endothelial vessels in the form of a lacy network separating maternal and fetal blood supplies. The junctional zone of compressed and aligned placental cone cells bounds the allantoidean placenta.

Just beyond the junctional zone is the vacuolated decidua basalis containing many giant cells and numerous large irregular maternal blood lacunae; it is more compact and uniform mesometrially except for interruptions by maternal blood vessels. That portion of the decidua basalis lying

against the circular and the longitudinal smooth muscle coats is compact.

As viewed in cross section, the uterine epithelium with its cavity has progressed around the uterus except for the main mesometrial area supplying blood to the placenta.

Sixteenth day of gestation

Membranes. All membranes are as on day 15 except for the visceral yolk sac, the villi of which are diminished in size.

Integral chorioallantoic and yolk sac placenta. Where the allantoidean placenta and yolk sac overlap there are maternal blood lacunae in direct contact with Reichert's membrane and the parietal yolk sac. Some of these maternal blood spaces are lined in part by trophoblastic endovascular plasmodial cells. The previously described condition of allantoidean

TABLE 1

MORPHOGENESIS OF THE VITELLINE AND ALLANTOIC PLACENTAE OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)

KEY O=NOT FORMED P=PRESENT L=LOST PL=LOST IN SOME AREAS ONLY									
VITELLINE PLACENTA								DAY	ALLANTOIC PLACENTA
MATERNAL	FETAL							REMARKS	
UTERINE ENDOTHELIUM	UTERINE STROMA	UTERINE EPITHELIUM	A TROPHECTODERM OR A REICHERT'S MEMBRANE	PARIETAL ENDOTDERM OR	ATTENUATED PAR. ENT.	VISCERAL ENDOTDERM	YOLK SAC SPL. MES. ENDOTHELIUM OF YOLK SAC SPL. MES.		
UTERINE ENDOTHELIUM	UTERINE STROMA	UTERINE EPITHELIUM	CHORIONIC ECTODERM	CHORIONIC SOM. MES. ENDOTHELIUM OF SPL. MES. OF ALLANTOIS					
P	P	PL	P	O	O	O	O		6
L	L	L	P	P	O	P	P		7
L	L	L	P	L	P	P	P		8
L	L	L	P	L	P	P	P		9
L	L	L	P	L	P	P	P		10
L	L	L	P	L	P	P	P		11
L	L	L	P	L	P	P	P		12
L	L	L	P	L	P	P	P		13
L	L	L	P	L	P	P	P		14
L	L	L	P	L	P	P	P		15
L	L	L	P	L	P	P	P		16

VISCERAL YOLK SAC IS VASCULAR
PARIETAL YOLK SAC IS VASCULAR

ALLANTOIS
APPROACHING SEROSA
THICK HEMOCHORIAL
THINNER HEMOCHORIAL
THINNEST HEMOCHORIAL
HEMOENDOTHELIAL
"
"
"
"

placenta penetration by the combined layers of visceral yolk sac, parietal yolk sac and Reichert's membrane is also present.

Uterus. In penetrating the sides of the mesometrium the uterine lumen has excavated under some of the allantoidean placenta, and caused it to protrude into the uterus as a flattened knob. The peripheral wall (uterine wall) of the re-established uterine lumen is lined with renewed columnar epithelium, but the medial or decidual wall is faced with a smooth-surfaced connective tissue selvage and lacks an epithelium. Mesometrial penetration by the uterine lumen forecasts the line of cleavage at parturition.

SUMMARY

1. The morphogenesis of the vitelline and allantoic placentae from day 6 to term (day 16) is described in the golden hamster.

2. On the 6th day post coitus the blastocyst has eroded the uterine epithelium of the implantation chamber. Day 7 is marked by the establishment of the placental cone, Reichert's membrane, and the invasion of the uterine stroma by trophoblast giant cells.

3. The entoderm forms both visceral and parietal yolk sacs on the 8th day and maternal blood lacunae bathe the ectodermal placental cone cells.

4. By the end of day 9, the visceral yolk sac is vascularized (the parietal is never vascularized) and the allantoic endothelium makes contact with the somatic mesoderm layer which in turn lies against the laminae at the base of the placental cone ectoderm. The extension of the allantoic vessels into the laminae establishes a hemochorial placenta. Antimesometrially the decidua capsularis is defined by the re-established uterine lumen.

5. The vascular visceral yolk sac convolutes on day 10 and projects villi into the yolk sac cavity.

6. On day 12 the hemochorial placenta becomes hemo-endothelial and integral with the vitelline placenta.

7. Reichert's membrane and the parietal yolk sac disappear in a circumscribed area antimesometrially on day 14½.

8. On day 16 (term) the allantoidean placenta flattens and separates from the basalis where the new uterine lumen is being re-established.

9. Table 1 presents in concise form the changes occurring from day to day in both the vitelline and allantoic placenta.

LITERATURE CITED

- BRIDGEMAN, JANE 1948 A morphological study of the development of the placenta of the rat. *J. Morph.*, 83: 61-86.
- 1948 A morphological study of the development of the placenta of the rat. II. An histological and cytological study of the development of the chorioallantoic placenta of the white rat. *J. Morph.*, 83: 195-224.
- GRAVES, ARTIS PARIS 1945 Development of the golden hamster, *Cricetus auratus* Waterhouse, during the first nine days. *Am. J. Anat.*, 77: 219-251.
- VENABLE, JOHN H. 1946 Pre-implantation stages in the golden hamster (*Cricetus auratus*). *Anat. Rec.*, 94: 105-120.
- WARD, MARGARET C. 1948 Early development and implantation of the golden hamster and associated endometrial changes. *Am. J. Anat.*, 82: 231-275.

PLATES

KEY TO ABBREVIATIONS

al, allantois	py, parietal yolk sac
am, amnion	R, Reichert's membrane
bl, maternal blood lacuna	st, stroma
e, embryo	tr, trophectoderm
ec, extraembryonic coelom	ue, uterine epithelium
icm, inner cell mass	ul, uterine lumen
jz, junctional zone	vy, visceral yolk sac
mbv, maternal blood vessel	yc, yolk sac cavity
pc, placental cone	

PLATE 1

EXPLANATION OF FIGURES

- 1 Implanted blastocyst at the end of 6 days, showing erosion of the uterine epithelium in the region of the blastocyst. $\times 516$.
- 2 Placental cone at the end of day 9. Allantoic blood vessels and maternal blood lacunae penetrate the cone. $\times 120$.
- 3 Embryonic membranes and placentae at the end of day 8. The deflection (arrow) marks the transition from visceral to parietal yolk sac. $\times 50$.
- 4 The laminae at the end of the 9th day. The advancing giant cells have segregated a portion of the stroma. $\times 50$.

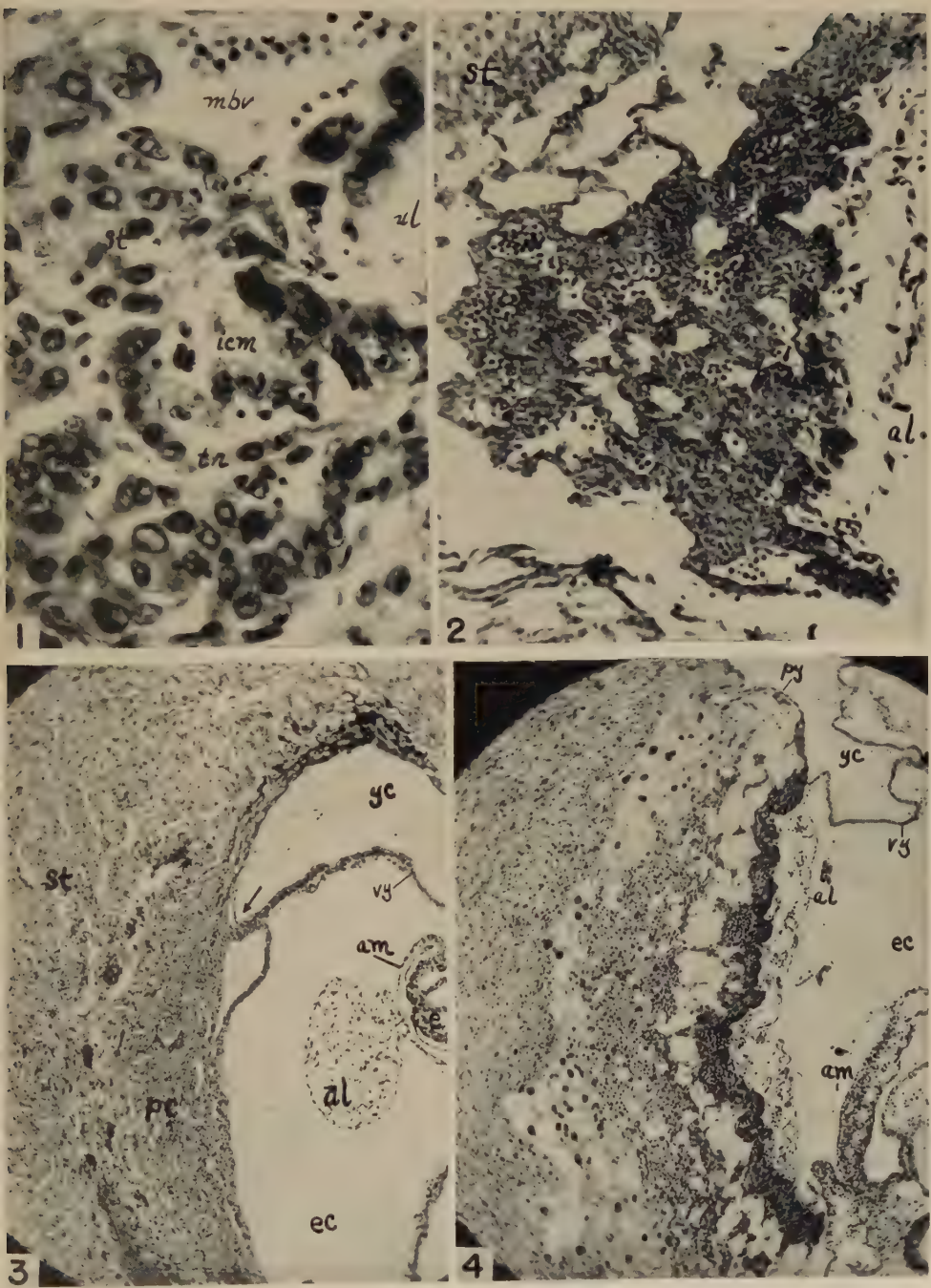


PLATE 2

EXPLANATION OF FIGURES

5 Antimesometrial region at the end of day 9. Maternal blood lacunae lie in apposition to Reichert's membrane and the parietal yolk sac. $\times 120$.

6 A portion of the hemochorial placenta on day 9. $\times 516$.

7 Antimesometrial region at the end of day 10. The re-established lumen has divided the basal is and defined the capsularis. The clear areas outside the uterine lumen are artifacts. $\times 120$.

8 Hemoendothelial placenta on day 12. The uniform grey is maternal blood in lacunae. Endothelial nuclei of the allantois are elongate. The fetal red blood cells present a black circular nucleus. Arrows point to select spots showing the hemoendothelial relation. The large nuclei belong to scattered trophoctodermal cells. $\times 516$.

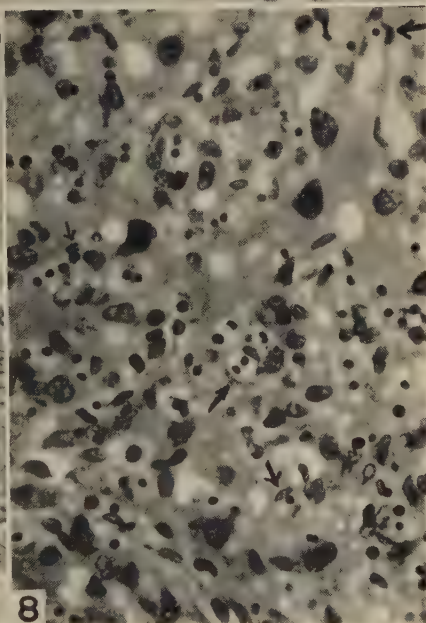
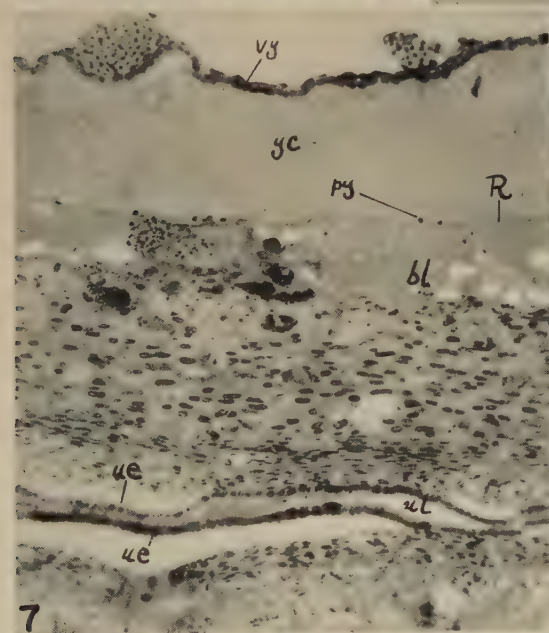
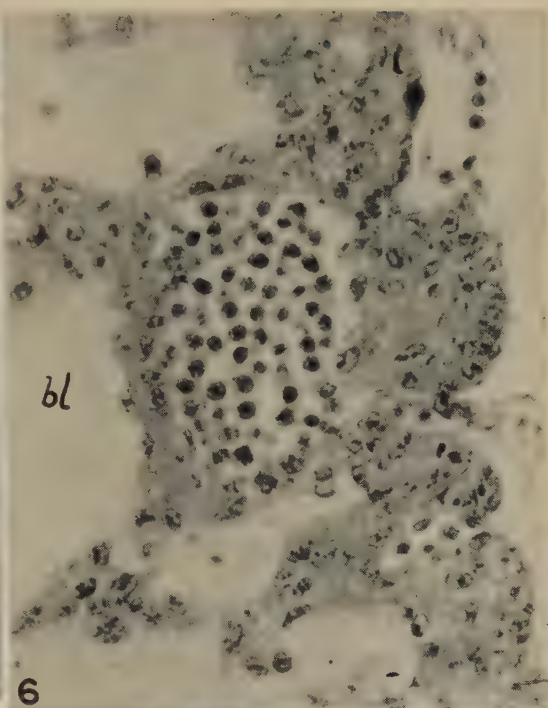
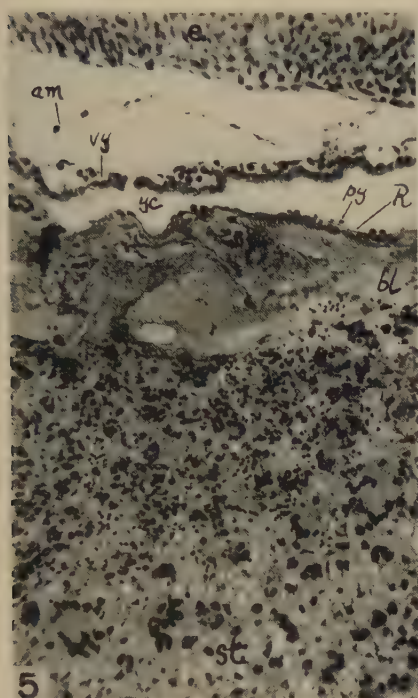


PLATE 3

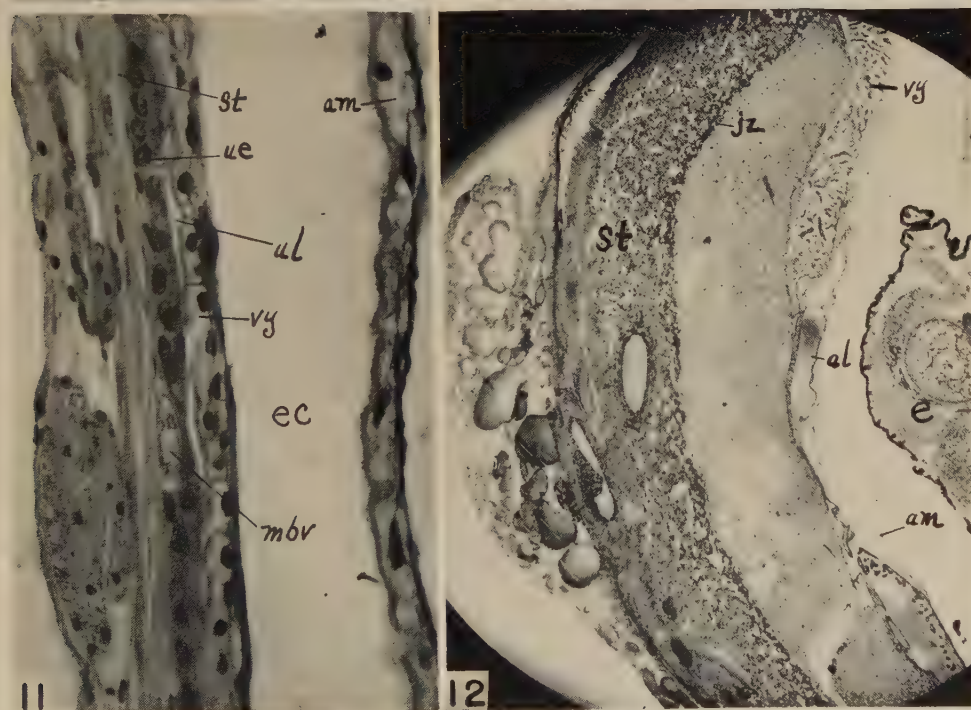
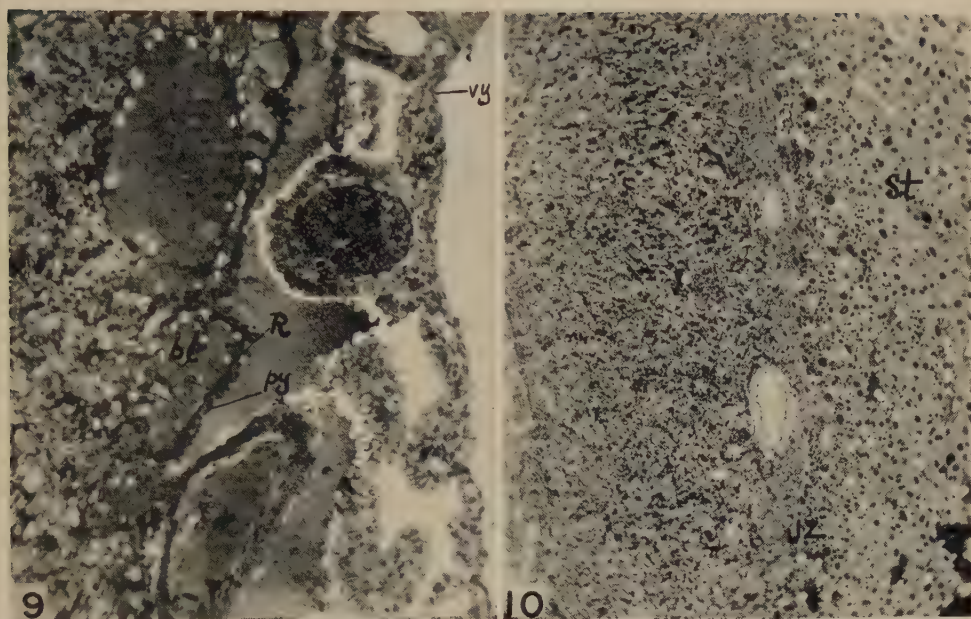
EXPLANATION OF FIGURES

9 Vitelline and allantoic placentae on day 13. Pools of maternal blood bathe Reichert's membrane and the apposed parietal yolk sac. The visceral yolk sac is thrown into convolutions and villi. $\times 120$.

10 Labyrinthian structure of the area of exchange in the allantoidean placenta at $14\frac{1}{2}$ days. Large allantoic blood vessels lie just inside the junctional zone. Giant cells are present in the decidua. $\times 50$.

11 Antimesometrial region at the end of the 15th day. The cuboidal epithelium of the visceral yolk sac forms the inner wall of the re-established uterine lumen (complete inversion). $\times 516$.

12 A topographic view of the allantoidean placenta at the end of day 15. $\times 10$.



HISTOLOGY OF THE SKIN AND HAIR OF THE ADULT CHINCHILLA ¹

HARRY H. WILCOX

*Department of Anatomy, School of Medicine, University of Pennsylvania,
Philadelphia*

ELEVEN FIGURES

INTRODUCTION

Specialization among animals is one of the most intriguing subjects confronting zoologists. Whenever naturalists gather, their conversation is apt to involve discussion of some form of animal whose morphological characteristics include several highly specialized features, about which the known facts are fewer than popular beliefs. It was in the course of such a conversation, centered around the habits of the Chinchilla, that a statement was made that led me to pursue and report the following study.

The Chinchilla is a hystricomorph rodent whose natural distribution is restricted to the Andes of Bolivia, Chile and Peru. This small squirrel-sized animal with long hind legs, long ears and long bushy tail bears one of the most beautiful coats of fur. The search for commercially valuable furs led to importation of this animal to this country many years ago, and ranchers have succeeded in maintaining and breeding the stock rather successfully. One rarely associates the word Chinchilla with anything but fur, and it is strange that no scientific study of the fur of the Chinchilla has been undertaken. Nowhere in the literature was I able to find reference to the arrangement or structure of the hairs in the Chinchilla skin. A single microscopic preparation in the Pierson Col-

¹Aided by a grant from Faculty Research Fund of the University of Pennsylvania.

lection of 1881-82 was the only specimen of *Chinchilla* hair in our laboratory, and it appears to contain contaminating hairs of other animals.

The *Chinchilla* has a unique arrangement of its hairs that seems to have been known but never reported scientifically. During the conversation mentioned above, it was stated that the *Chinchilla* has as many as 80 hairs emerging through a single pore of the skin. This seemed almost unbelievable and I questioned it. None but hearsay evidence was to be had and I remarked that I would like to examine the skin and hair histologically to determine the actual condition existing. Therefore, a specimen was offered for examination, and after this investigation was begun I soon found proof that the above statement is very close to the truth (fig. 3).

MATERIAL AND METHODS

Through the kindness and generosity of Mr. Wallace Greer of New Market, Va. and Mr. William Craighead of Harrisburg, Pa., one living adult specimen and one preserved in 10% formalin were made available to me for this study.

Biopsied material from the living specimen was immediately fixed in Bouin's solution, imbedded in paraffin, serially sectioned at 10 μ , and stained with haematoxylin and eosin. Pieces of the formalin-fixed skin from the preserved specimen were handled similarly. Samples of skin and hair were taken from the dorsum and ventrum of the trunk, the tail, the pinnae and the cheeks. Sections were cut longitudinally and transversely in respect to the hairs.

More recently a fetus of 105 days (gestation period in the *Chinchilla* is 111 days) was sent to me by Mr. Craighead. A still-born and one specimen that died at two months post natum were given by Mr. Jack Trout of Wayne, Pa. There are striking differences between fetal and adult hair, but more material is required before a study of the ontogenetic development of the hairs and hair pattern can be completed. The condition in the adult will form the main portion of

this paper, however a few notes on the fetus and newborn will be included.

DESCRIPTION OF OBSERVATIONS

The epidermis of the adult is composed of three recognizable layers: stratum germinativum, stratum granulosum and stratum corneum. The stratum germinativum is only two cell layers thick and its cells are irregular in shape, ranging from polyhedral to flattened. Cell boundaries are rather indistinct but the nuclei are large and ovoid.

Above the stratum germinativum is a single, much flattened cellular layer, the stratum granulosum. The flattened cells of this layer have degenerate nuclei and contain fine irregular cytoplasmic granules. A thick stratum corneum of flattened, non-nucleated cornified cells forms the outermost layer. No stratum lucidum is found between granulosum and corneum.

The corium is a thick connective tissue layer whose inner boundary is unrecognizable except in regions where the panniculus carnosus or other integumentary muscles or organs occur. There is no definite papillary zone.

The epidermis of the fetus, newborn and two months old specimen resemble that of the adult. However, the stratum granulosum is quite prominent and the stratum corneum is somewhat thinner than that of the adult.

The arrangement of the hair over the body of the adult is quite uniform. Except on the cheeks, tail and pinnae, hairs occur only in dense clusters. The clusters (figs. 1, 2, 7) usually consist of a central guard hair and two outer groups of smaller wool hairs. All hairs of a single cluster emerge from the skin through one common pore which is partially subdivided by the epidermal cells around the guard hair. Most hairs on the cheeks have this arrangement, but long heavy sensory hairs, the vibrissae, are also present (fig. 5). The vibrissae are independent hairs and are completely separate from one another. The hairs on the tail are separate and independent in respect to their emergence, although they

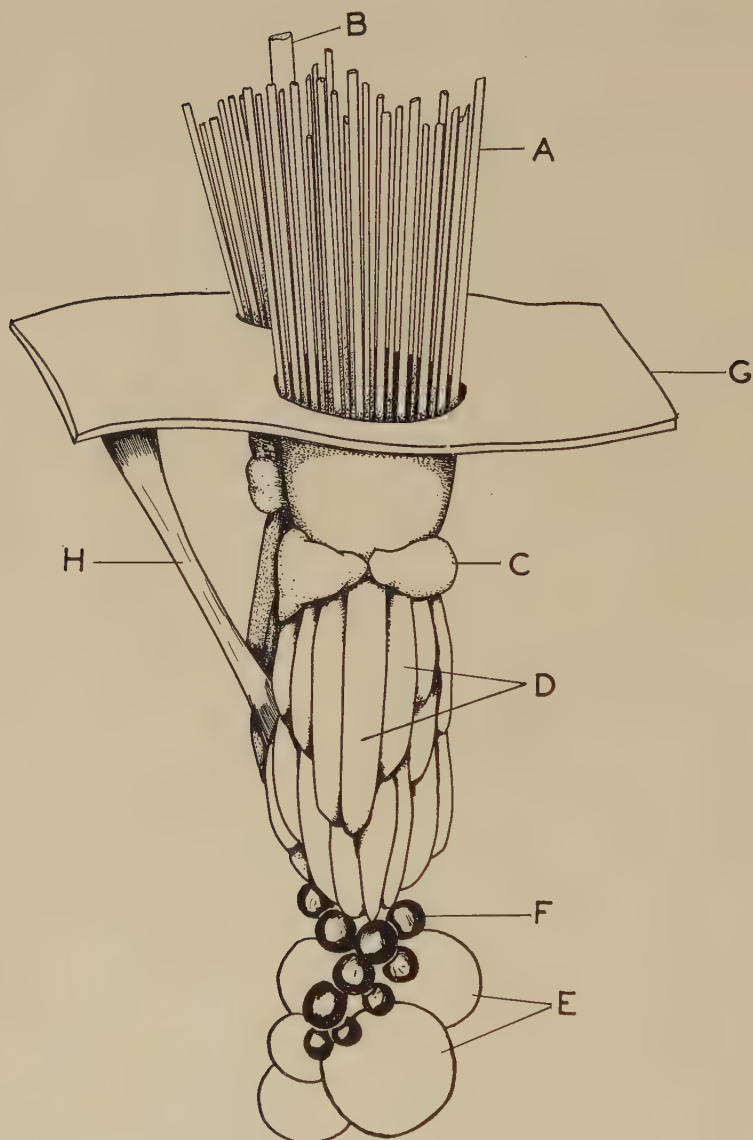


Fig. 1. Semi-schematic diagram of a hair group of adult.

- | | |
|---------------------|--------------------------|
| a. Wool hair. | e. Fat cells. |
| b. Guard hair. | f. Pigmented cells. |
| c. Sebaceous gland. | g. Epidermis. |
| d. Follicles. | h. Arrector pili muscle. |

are arranged in small groups of 3 to 8 hairs (fig. 6). The hairs on the pinnae are separate and are not arranged in groups.

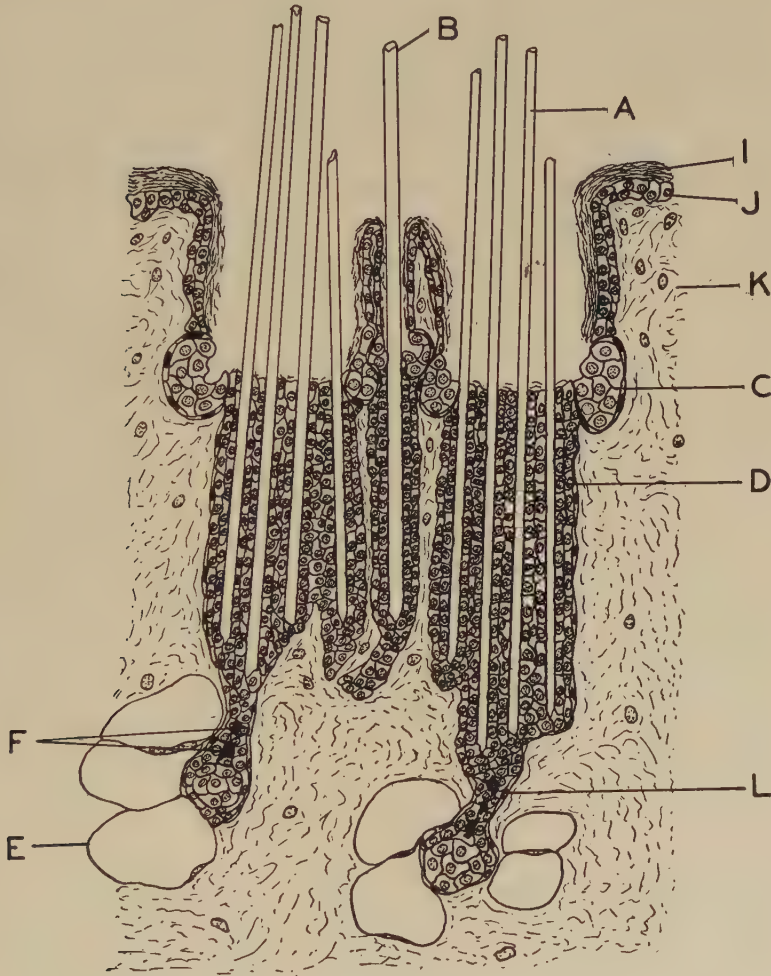


Fig. 2 Diagram of a longitudinal section of a hair group of adult.

- i. Stratum corneum.
- j. Stratum germinativum.
- k. Corium.
- l. Proliferating follicle.

Other labels as in figure 1.

The softness and denseness of Chinchilla fur is due to the fineness and clustering of the hairs. Guard hairs (large hairs located centrally in the clusters) range in size from 12μ to 15μ in diameter. There is usually only one of these in each cluster, although as many as three do occur occasionally. The wool hairs of the outer groups range in size from 5μ to 11μ in diameter. The number of hairs emerging from a single opening in the skin is variable and appears to be generally in the range of 50 to 60. The maximum number I was able to count was 75 (fig. 3).

A single arrector pili muscle is attached to each of the clusters of hairs (fig. 1). The muscle runs obliquely downward between the two lateral groupings and attaches around the base of the central guard hair. Each guard hair has its own sebaceous gland; the outer groups of wool hairs are served by 3 or 4 glands per group. All sebaceous glands open at the same level into the base of the hair pore (figs. 2, 4).

The three layers of the epidermis are reflected downward from the surface of the skin to form the pore. The outer layers become thin and disappear near the base of the pore around the openings of the glands. The stratum germinativum continues downward around each of the hairs to form a compound follicle. This stratum is unmodified and cannot be subdivided into the various layers of a typical hair follicle of human skin. Two cell layers surround each of the guard hairs and these differ only in size and shape of nuclei. The inner cell layer around the hair is made up of cuboidal cells with spheroid nuclei. The outer layer is formed by low columnar cells with larger ovoid nuclei. Around the wool hairs of the lateral groups no layering is evident. A single cell layer may exist between two hairs and serve as a follicle wall for both (fig. 11). No evidence of layering is apparent at any level of the follicle proper and no bulb formation is to be found at the base of the hairs (fig. 4). At the proximal ends of the follicles are found strands of proliferating cells, many of which are pigmented. These strands apparently give rise to new follicles.

In the vibrissae, the structure of the follicle is quite different from that of the body hair and resembles a typical hair follicle of other species. Its structure is illustrated in figure 8.

Although vibrissae possess typical bulb and papilla, these structures are not found at the base of the hairs of the body or on the tail, even though follicles of the tail may give rise to individual hairs. In the tail, 4 or 5 layers of cells continuous with the stratum germinativum make up the only layers of the follicle. A similar arrangement exists in the hair follicles of the pinnae. The chief difference between follicles of the body and those of tail and pinnae is the clustering of finer hairs from the former.

In the fetus the hairs are independent of one another. Each has its own sebaceous gland and arrector pili muscle, and exits through its own pore (fig. 9). The follicles have the typical layering, bulbs and papillae (fig. 10). The hairs of the newborn are arranged in clusters of 8 to 20 hairs. These are divided into two or three groups per cluster—a guard hair not being present in all groups. Sebaceous glands are present around each group and open as in the adult at the base of the hair pore. A single arrector pili muscle is attached to each cluster. Follicular layers, bulbs and papillae are recognizable. By the second month post natum the hair pattern is quite similar to that of the adult, although bulbs and papillae are evident.

SUMMARY

The general body hair of the Chinchilla is uniformly arranged in dense clusters of as many as 75 hairs. Each cluster typically consists of a single guard hair and two lateral groups of wool hairs. All the hairs of a single cluster emerge through a common pore. Each cluster of hairs has a single arrector pili muscle. Guard hairs have their own sebaceous glands, while 3 or 4 other glands serve all the hairs of a lateral group.

Independent hairs are found only as vibrissae or on tail and pinnae. Follicular layering is found only in the vibrissae.

The dense clusters of hairs in the adult Chinchilla are in marked contrast to the independent hairs found in the 105 days fetus. The transition from the fetal state to that of the adult occurs before the second month post natum.

PLATE 1

EXPLANATION OF FIGURES

- 3 Single cluster of hair from dorsum. $\times 3.5$.
- 4 Transverse section of skin of dorsum, showing part of wool hair group.
 $\times 160$.
- a. Arrector pili muscle.
 - b. Sebaceous gland.
 - c. Pigmented cells.
- 5 Transverse section of cheek vibrissa. $\times 60$.
- a. Medulla.
 - b. Cortex.
 - c. Inner epithelial sheath.
 - d. Outer epithelial sheath.
 - e. Connective tissue sheath.
 - f. Venous plexus.

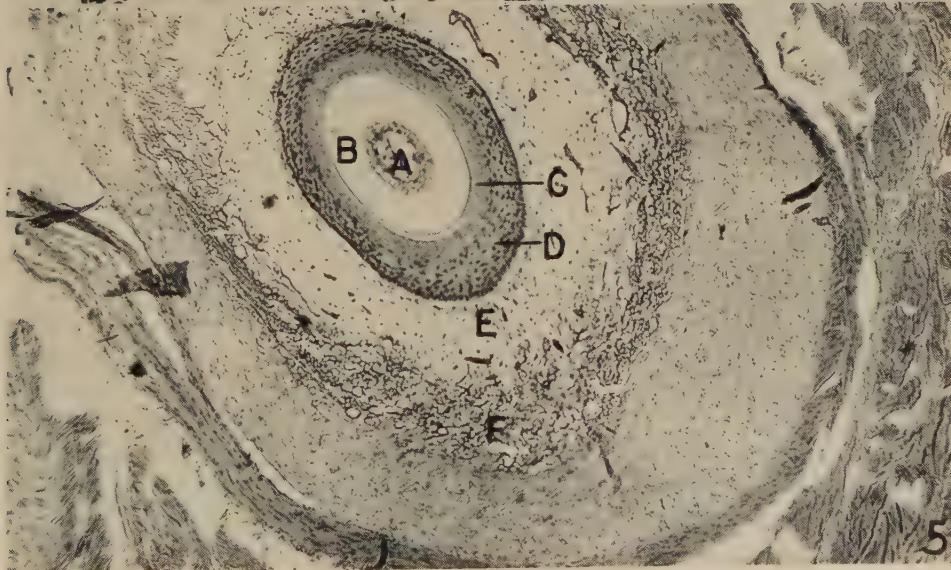
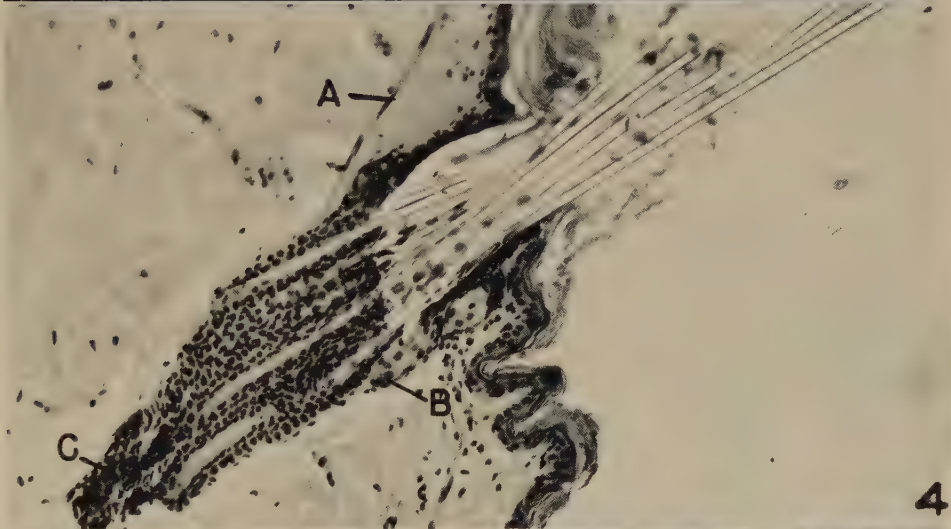


PLATE 2

EXPLANATION OF FIGURES

- 6 Frontal section of skin of tail. $\times 116$.
- 7 Frontal section of skin of dorsum. $\times 125$.
 - a. Guard hair.
 - b. Wool hair.
- 8 Transverse section of cheek vibrissa. $\times 1020$.
 - a. Cortex.
 - b. Inner epithelial sheath.
 - c. Outer epithelial sheath.
 - d. Connective tissue sheath.

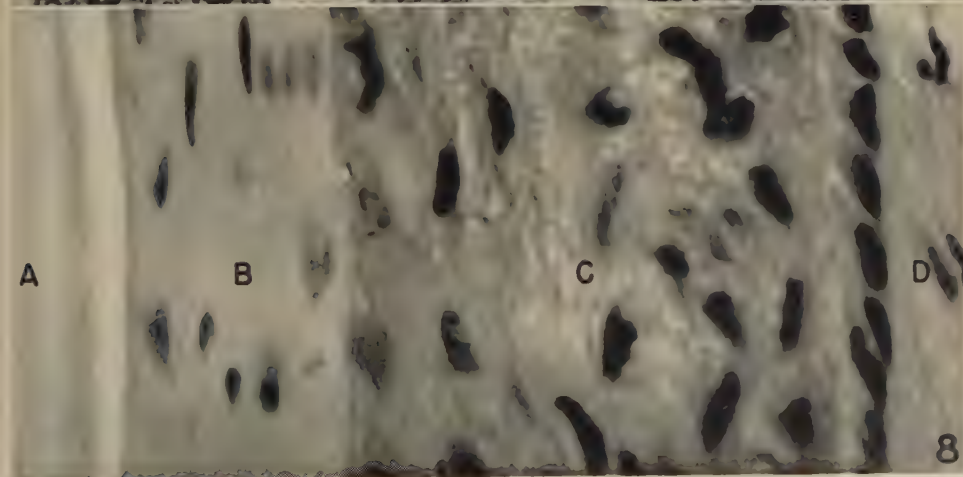
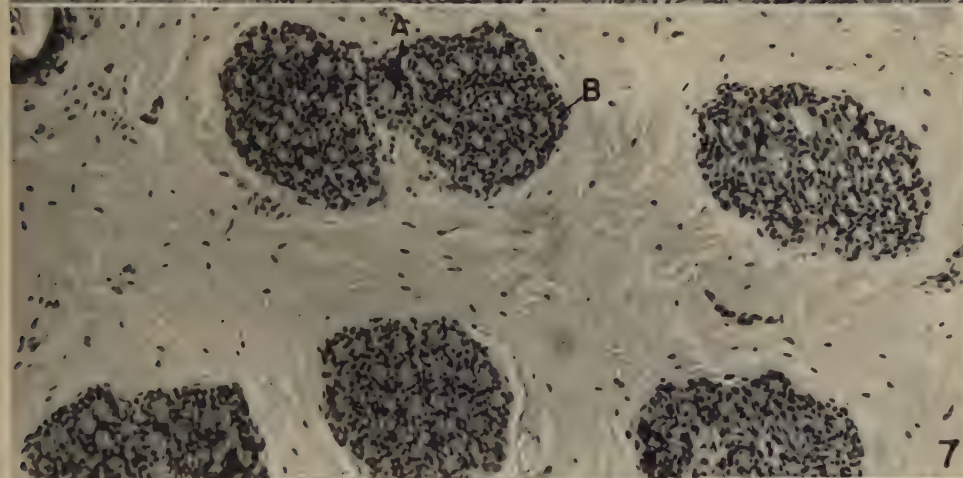
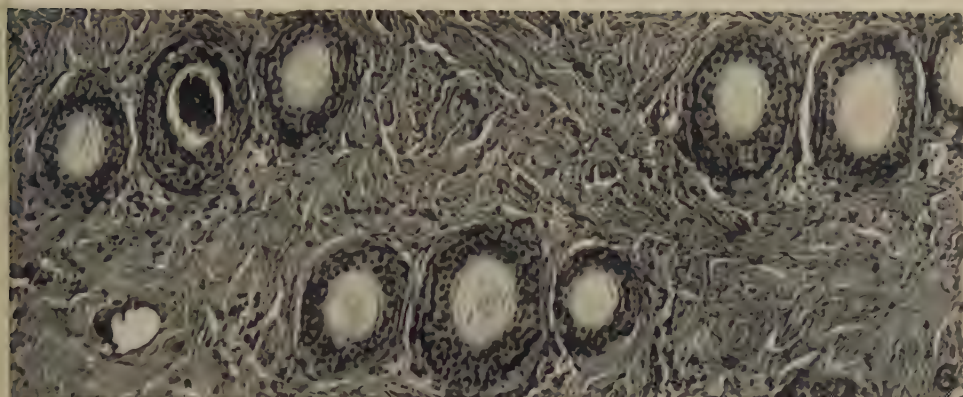
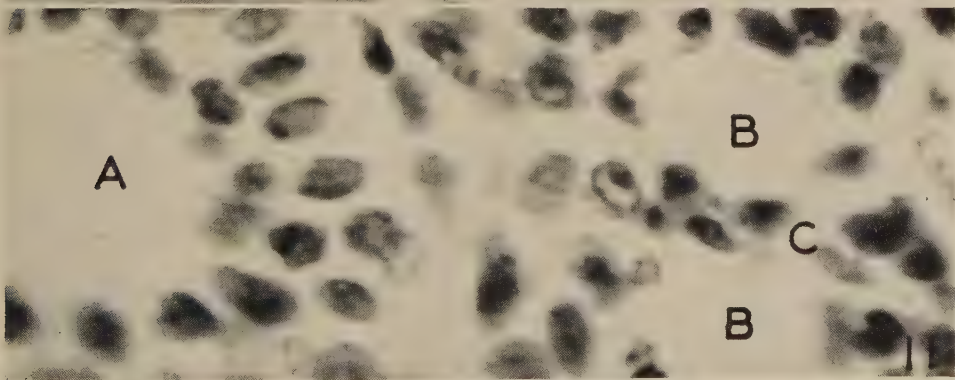
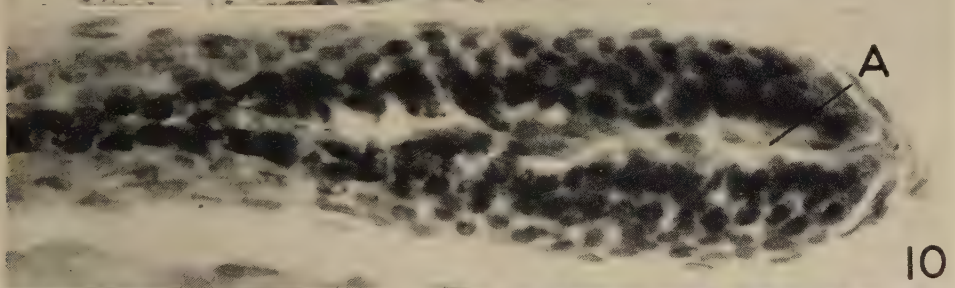
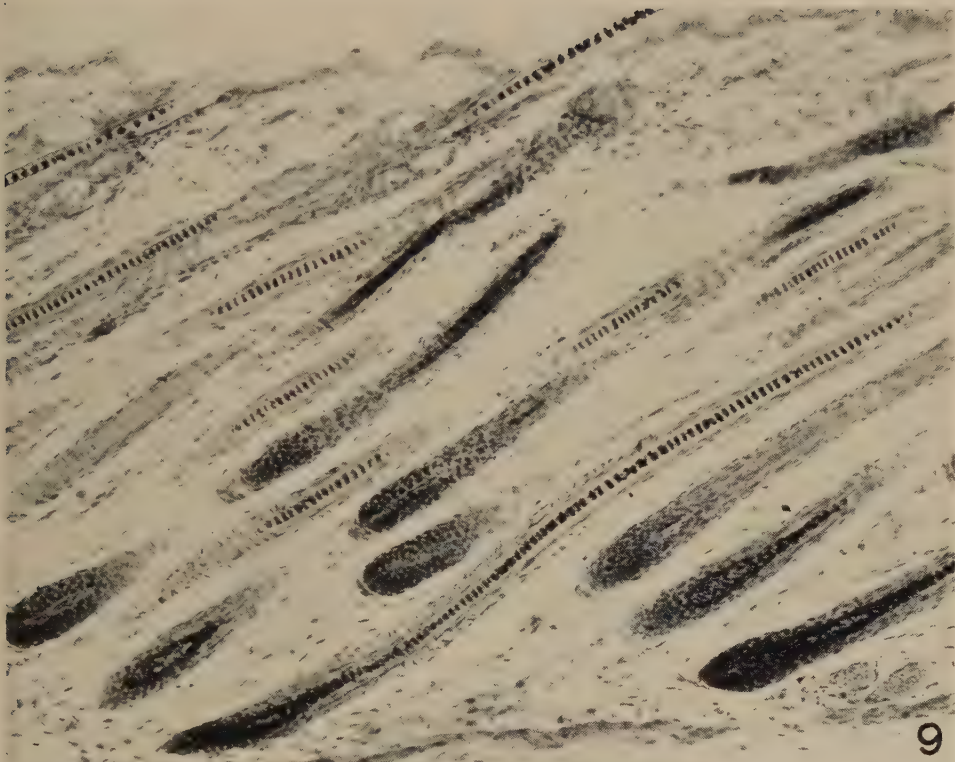


PLATE 3

EXPLANATION OF FIGURES

- 9 Transverse section of skin of dorsum of 105 days fetus. $\times 150$.
- 10 Hair bulb of 105 days fetus. $\times 750$.
a. Papilla
- 11 Transverse section through portion of hair cluster of dorsum of adult to show arrangement of follicle cells.
a. Guard hair.
b. Wool hair.
c. Follicle cells.



THE MORPHOLOGY OF THE ATTACHMENT BETWEEN THE DERMIS AND THE EPIDERMIS ¹

GEORGE F. ODLAND

Department of Anatomy, Harvard Medical School, Boston, Massachusetts

TWELVE FIGURES

INTRODUCTION

The structure of the junction of the dermis and epidermis has been a subject of dispute and conjecture for over 50 years. In 1916 Herxheimer reviewed the earlier literature which contained a variety of opinions as to the nature of the "basement membrane" of the epidermis. He concluded that a translucent membrane existed and that it was intimately associated with fine epithelial fibers projecting toward the dermis. Four years later Frieboes ('20) introduced the concept of a "Verfilzungszone" consisting of tangled argyrophilic reticulum fibers lying between the epidermis and the corium. It was his belief that cytoplasmic processes of the basal cells of the epidermis entered this zone. Homma ('22) described many of the reticulum fibrils as terminating in blunt or bulbous endings associated with the basal processes of the epidermal cells. Subsequent investigators (Kogoj, '23; Hoepke, '25; Pautrier and Woring, '30; Manganotti, '30) concerned themselves with the intimate relationships between the reticulum fibrils and the projections of the basal epidermal cells. Blunt endings of reticulum fibers, which were often noted, were designated as Homma's bodies. Szodoray ('31), after reviewing the accumulated evidence, concluded that the

¹ This work was supported in part by funds received from the Eugene Higgins Trust.

basal epidermal processes interdigitated with dermal reticular fibrils much like "two brushes fitted together along their bristle surfaces." However, Dick ('47), in the most recent discussion of this subject, points out that it is unproved whether processes of the epithelial cells fit into an open reticular meshwork or whether the basal cytoplasm of the epithelial cells is somehow affixed or penetrated by free ends of reticulum fibrils.

Thus, from examination of the literature, it is apparent that there is lack of agreement on some points regarding the structure of the junction of the epidermis and dermis. In view of this, the present study was undertaken to establish more clearly, if possible, the exact morphological relationships of the basal cells of the epidermis to the underlying dermis. Sections of skin cut perpendicularly as well as parallel to the skin surface and stained by Masson's method and Pap's silver technique for reticulum formed the basis of the investigation.

MATERIALS AND METHODS

Pieces of abdominal human skin were obtained from two adult men at surgical operation, as well as from the plantar surface of an amputated toe of an adult woman. Pieces of skin from palm, sole, abdomen and medial aspect of the thigh were obtained from three young rhesus monkeys. The specimens were fixed immediately in Zenker's fluid or Rossman's fluid (absolute alcohol, formalin and picric acid), and were spread upon stiff paper during fixation so that distortion was kept at a minimum. The blocks were embedded in paraffin. Sections of 6μ thickness were cut perpendicularly as well as parallel to the skin surface. The sections were stained by Masson's trichrome mixture (acid fuchsin, orange G and light green), and with Pap's ammoniacal silver nitrate method (as modified by Mitchell and Wislocki, '47) for the demonstration of reticulum fibers. Mallory's connective tissue stain, hematoxylin and eosin, and eosin and methylene

blue stains were tried but rejected in favor of the two methods cited.

OBSERVATIONS

The morphology of the basal surface of the epidermis. The basal surface of the epidermis is characterized by irregular ridges and cones ("rete pegs") of epidermis projecting toward the dermis. Between adjacent ridges lie the so-called dermal papillae, molded against the overlying epidermal contours. The number of epidermal ridges and cones per unit area as well as the depth and breadth of these structures differs in various regions of the integument. Sections through the plantar skin of a human toe and the skin of the thigh of a monkey (figs. 2 and 3) illustrate these differences; the relative prominence of the epidermal ridges in skin from the toe is evident.

The basal epidermal cells are generally of a low columnar type and are provided with basal cytoplasmic processes projecting into the dermal connective tissue and oriented in the direction of the long axes of the cells. The shape and extent of the basal processes are most clearly revealed in sections of vertically cut palm or sole stained with Masson's trichrome mixture (fig. 1 a). Some degree of variation is evident in the length and thickness of these fingerlike cytoplasmic extensions, a number of which are branched.

In order to understand more fully the arrangement of the epithelial processes, serial, horizontal sections placed through the basal layer of the epidermis were examined. Figure 11 is a photograph of a horizontal section of the skin of the palm of a monkey, the 4 small rectangles in this figure corresponding to the 4 drawings at higher magnification shown in figure 1 b, c, d and e. The shapes of the basal epidermal cell have been drawn at 4 cross-sectional levels (figs. 1 b, c, d and e) from the nucleus to the tips of the epithelial processes. For the purpose of orientation, the approximate level of each of the drawings in figures 1 b, c, d and e is indicated by a line of reference at the side of figure 1 a. In figures 1 b and c the intercellular bridges characteristic of the epidermis are

visible between adjacent cells. Closer to the dermis, in figures 1 d and e, the individual cell outlines are not distinguishable; at these levels merely the cross sections of the cell processes are visible with homogeneous, green-staining material between them. As seen here in cross section, the processes have a distinctly angular appearance, much as if they had acquired their shapes by being molded by a net or meshlike structure.

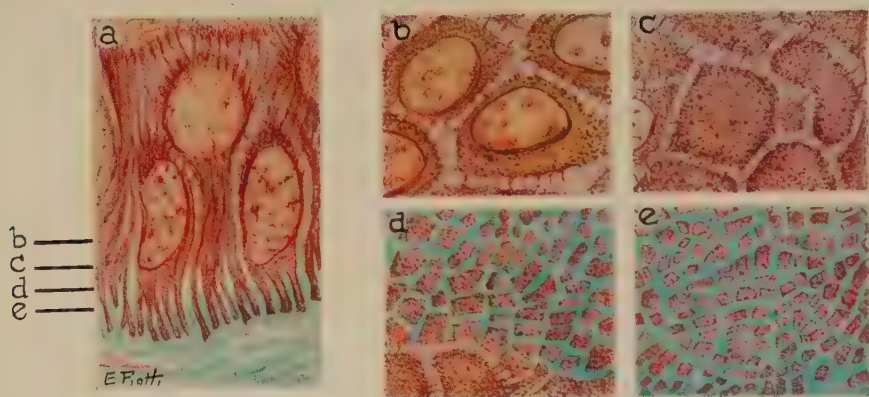


Fig. 1 Drawings of vertical and horizontal sections of skin from the palm of a rhesus monkey illustrating significant morphological details of the basal epidermal cells. Figure 1 a, drawn from a vertical section, demonstrates the fingerlike appearance of the basal processes of the basal epidermal cells. Observe that some of the processes appear to branch or divide. Figures 1 b, c, d, and e show the cross-sectional appearances of the basal epidermal cells (b and c) and their processes (d and e) at 4 successive levels (indicated by horizontal lines in fig. 1 a) from the nucleus to the tips of the processes. In b and c intercellular bridges are visible, and in d and e the angular cross-sectional outlines of the basal processes are evident. Between the processes homogeneous, green-staining material is present. The rectangles in the section of skin shown in figure 11 indicate the locations. Zenker's fluid. Masson's trichrome stain. Approx. $\times 2000$.

At level d the area of the cross section of a single process is distinctly greater than at level e, located nearer the tips of the processes; at the same time a greater number of individual processes are seen per unit of area at level e than at level d, indicating that the processes branch as they penetrate the dermal tissue. Several such branching processes are actually visible in the vertical section shown in figure 1 a.

In the skin of the abdomen and thigh the ridges of the basal surface of the epidermis and the corresponding dermal papillae are less numerous and more shallow than in the palm or sole (cf. figs. 2 and 3). The basal epithelial processes are also correspondingly shorter and less numerous, and branching is less evident.

The morphology of the subepidermal reticulum of the dermis. Immediately beneath the basal surface of the epidermis lies a tangled meshwork of argyrophilic reticular fibrils continuous with the underlying collagenous fiber bundles. The network of reticular fibers beneath the basal epidermal cells is selectively and sharply impregnated by silver stains, whereas the cytoplasm of the basal epidermal cells is poorly differentiated with only the nuclear outlines distinctly visible. Figures 2 and 3 illustrate this layer of argyrophil reticulum between dermis and epidermis in the thigh of a monkey and in the plantar surface of a human toe.

Closer examination of vertical sections of the skin from the sole or palm discloses an apparent variety of arrangements of the reticulum. The reticular fibrils at the bottom of the troughs enclosing the epidermal ridges give the appearance of being compressed into an intensely silvered, flattened mesh (fig. 6). From this reticular structure occasional argyrophilic digitations or dentations appear to penetrate above the lowermost projection of the basal epidermal cells. On the other hand, in the apices and to a lesser extent at the sides of the dermal papillae, the reticulum presents other pictures. At the apex of a papilla, long, coarse argyrophilic strands arise from the tangled subepithelial reticulum and appear to penetrate the cytoplasm of the basal cells (fig. 4), in many instances appearing to end in blunt, hooked, or bulbous tips (figs. 9 b, c). Careful examination of these strands reveals that they consist of delicate reticular fibrils gathered together in bundles. Moreover, it seems likely that the appearance of free endings of the argyrophil fibers within the epidermis is the result of strands arching obliquely through the tissue section and cut by the microtome blade at different

points along the length of the arches. This concept is illustrated in figure 8 in which three such arching strands are shown diagrammatically as they would appear to the eye at three different angles (a, b and c). According to the present interpretation, the photomicrographs in figures 9 a, b and c illustrate the actual appearances of such strands viewed in histological sections.

Finally, in places where the plane of the cut falls tangentially between the dermis and the epidermis, the reticulum has a still different appearance. Here, the fibers have a semblance of weaving irregularly for a considerable distance into the adjacent layers of epidermal cells. This seemingly deep penetration of the epithelium by the reticulum is more apparent than real, because, by focusing successively at different levels in a given field, it is apparent that the fibers never actually extend more deeply into the cells than to the level of the nucleus.

In sections of the skin of the thigh (figs. 5 and 7) the reticular fibers are similarly arranged, although the topographical differences are not so pronounced as in the skin of the palm or sole.

Serial horizontal sections of skin of the palm of a monkey impregnated with silver provide further information regarding the arrangement of the reticular meshwork. These sections reveal preponderantly a network of coarse reticular fibers (fig. 12). This network is most clearly seen in regions where the plane between epidermis and dermis lies nearly parallel to the plane of the section. Figure 10 shows in detail the arrangement in such a region; this figure consists of three optical sections at successive levels. Figure 10 a lies at the level of the nuclei of the basal epidermal cells, whereas 10 b is at a plane approximately 2μ beneath 10 a and shows a coarse network of reticular fibrils located immediately beneath the nuclear level. This network coincides approximately in shape and pattern with the homogeneous, green-staining substance seen between the basal epidermal processes in the sections stained by the Masson procedure (fig. 2 d). The

third optical section (fig. 10 c), situated a further 2μ toward the dermis, demonstrates the presence of a more delicate but more closely woven meshwork of reticular fibrils. In this instance the corresponding Masson-stained section is illustrated in figure 4 e, in which the brown-stained cell processes are more numerous and individually smaller than at the preceding level. The basic pattern of the reticular fibers as seen in horizontal sections of the monkey's palm is also evident in similarly cut sections prepared from the skin of the abdomen of man and monkey.

In reference to the features studied, no significant differences were encountered in human and monkey skin taken from comparable regions.

DISCUSSION

Dick ('47) reviewed the current concepts of the structure of the junction of the dermis and the epidermis, adding observations of his own on the character and presence of reticular and elastic fibers in that region. Regarding the role of the reticulum he concluded, "whether the fibrils form an open meshwork spread around the tips of the papillae into the spaces of which the bases of the epithelial cells fit, or whether they form a series of fibrils running up to the cells and fixing them, either by entering them or by filling the spaces between their basal processes is difficult to determine." Concerning elastic fibers, Dick decided that they do not take part in the ultimate junction between dermis and epidermis.

The solution of the problem of the arrangement of the reticulum depends upon the demonstration of a reticular pattern which can satisfactorily account for all of the histological appearances observable at the junction between dermis and epidermis. The preparations described in the present investigation demonstrate that the reticulum forms a continuous meshwork of delicate fibrils. Where the meshwork is penetrated by basal epithelial processes, the constituent reticular fibrils are compacted to form a network of coarse strands compressed between adjacent cell processes. When cut in

histological sections at certain angles, the arching strands of this meshwork appear in some situations as blunt or bulbous ends. The present analysis indicates, however, that this seeming appearance of free ends is actually the visual effect of an end-on view of arching bundles of reticular fibers oriented at right angles to the plane of the histological section. This interpretation would explain also the fibrillar processes of reticulum described by Szodoray ('31) as interdigitating with the processes of the basal epithelial cells.

In various regions of the body the skin is adapted to varying external forces. In so-called "pressure areas," as, for example, palm and sole, the junctional zone between dermis and epidermis is adapted to meet the variable shearing forces to which the skin is exposed. In such regions this adaptation is probably reflected by the relatively long basal epidermal processes as well as by the extensive development of the reticular net between the cell processes. Moreover, it is apparent that in such regions the epidermal ridges and cones attain a great depth. These morphological arrangements probably provide greater mechanical cohesion between the dermal and epidermal surfaces. In skin areas such as the abdomen and thigh the epidermal ridges and dermal papillae are lower and smaller and concomitantly the union of the epithelial cells with the dermal reticulum is less distinctive.

The amorphous ground substance present in the intercellular and interfibrillar spaces at the dermo-epidermal junction is not specifically brought out by the methods used in the present study, and consequently it has not been particularly described.

SUMMARY

The structure of the junction of the dermis and the epidermis was investigated in pieces of skin from man and rhesus monkeys, removed from palm, sole, thigh and abdomen. The skin was sectioned in both vertical and horizontal planes, and the sections were stained with Masson's trichrome stain and Pap's silver impregnation method for reticulum. It was concluded from study of the present material

that the cytoplasmic processes of the basal epidermal cells fit into the spaces of a continuous meshwork of dermal reticulum. The concept is advanced and proof offered that those reticular fibers which seem to terminate in bulbous ends in contact with the basal epithelium are in reality strands of the dermal reticular network which have been cut in such a way that they appear as blunt processes in the histological sections. Actually the reticulum possesses no free endings interdigitating with the cell processes, but consists, instead, of a continuous fibrillar mesh into the interstices of which the processes of the basal epithelial cells project.

LITERATURE CITED

- DICK, J. C. 1947 Elastic tissue of the skin. *J. Anat.*, 81: 201-211.
- FRIEBOES, W. 1920 Beiträge zur Anatomie und Biologie der Haut. II. Basalmembran: Bau des Deckepithels (Physiologische und pathologische Ausblicke). *Derm. Zeitschr.*, 31: 57-83.
- HERXHEIMER, K. 1916 Ueber die Epidermale Basalmembrane. *Derm. Zeitschr.*, 23: 129-134.
- HOEPKE, H. 1925 In: von Möllendorf's Handbuch der mikroskopischen Anatomie des Menschen. III/I, 33-35.
- HOMMA, H. 1922 Gitterfasern in normaler menschlicher Haut. *Wien. klin. Wochenschr.*, 35: 149-150.
- KOGOJ, FR. 1923 Über die Art der Verbindung zwischen Epidermis und Kutis. *Derm. Zeitschr.*, 39: 203-212.
- MANGANOTTI, G. 1930 Osservazioni sulla così detta membrana basale e sul tessuto reticolare del derma nell cute normale ed in alcune dermatosi. *Ricerche sui rapporti dermo-epidermica. G. Ital. Derm. Sif.*, 71: 1901-1916.
- MITCHELL, A. J., AND G. B. WISLOCKI 1944 Selective staining of glycogen by ammoniacal silver nitrate: a new method. *Anat. Rec.*, 90: 261-266.
- PAUTRIER, L. M., AND F. WORINGER 1930 Contribution a l'étude de l'histo-physiologie cutanée; les rapports morphologiques entre l'épiderme et le derme. *Ann. dermat. syph. Paris*, 1: 985-1005.
- SZODORAY, L. 1931 The structure of the junction of the epidermis and corium. *Arch. Derm. Syph. New York*, 23: 920-925.

PLATE 1

EXPLANATION OF FIGURES

2-7 Vertical sections through various pieces of skin fixed in Rossman's fluid and stained by the Pap's ammoniacal silver nitrate method for the demonstration of reticular fibers.

2 Skin from the plantar surface of a human toe illustrating the contours of the basal surface of the epidermis as characteristically seen in skin of the palm or sole. The delicate layer of blackened argyrophilic fibers is shown in contact with the basal surface of the epidermis. $\times 110$.

3 Skin from the medial surface of the thigh of a monkey showing the contours of the basal surface of the epidermis as well as the thin black layer of subepidermal reticulum. A comparison of figures 2 and 3 illustrates the relatively extensive development of epidermal ridges or "rete pegs" in the plantar skin as opposed to the thinner skin of the thigh. $\times 110$.

4 The long reticular strands visible near the apex of a dermal papilla in skin from the plantar surface of a human toe. The "x" in figure 2 indicates a location where such a reticular pattern regularly occurs. $\times 2200$.

5 The short rod-like appearance of the reticulum near the apex of a dermal papilla in skin from the thigh of a monkey. The "x" in figure 3 points to an area where this pattern occurs. $\times 2200$.

6 Flattened densely staining reticulum as it appears beneath an epidermal ridges of a human toe. The arrow in figure 2 indicates a point along the basal epidermal surface where such a reticular pattern prevails. $\times 2200$.

7 The reticular layer beneath the epidermal ridges from the skin of a monkey's thigh. The arrow in figure 3 indicates a typical location in which this appearance is seen. The similarities of detail between the regions illustrated in figures 5 and 7 are evident. $\times 2200$.

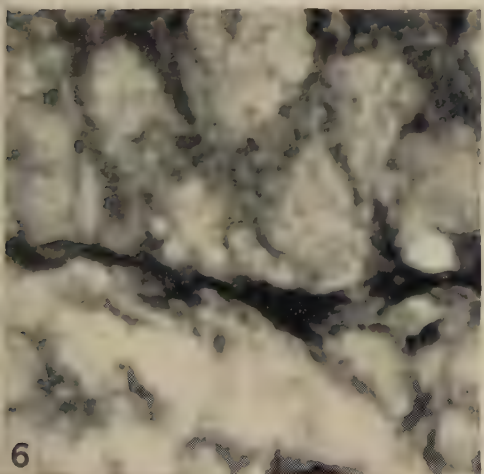
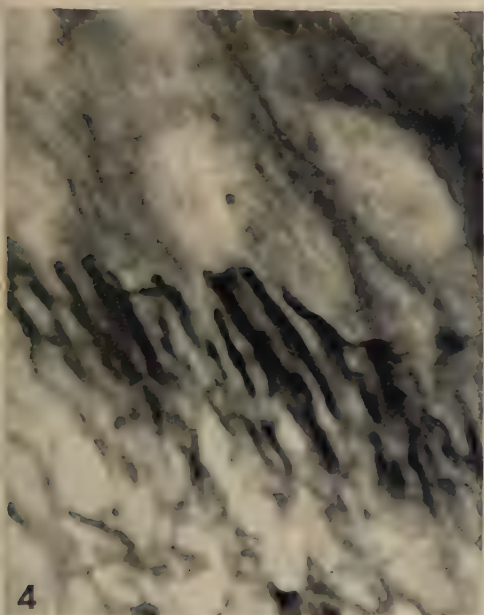
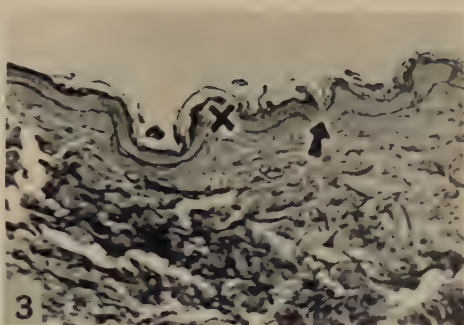
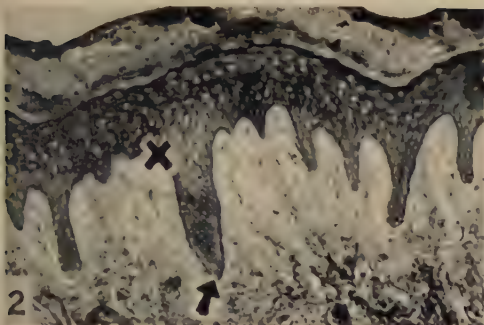
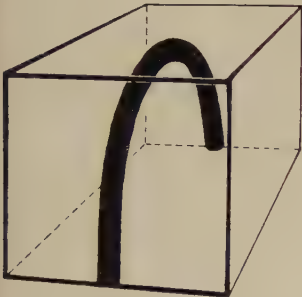


PLATE 2

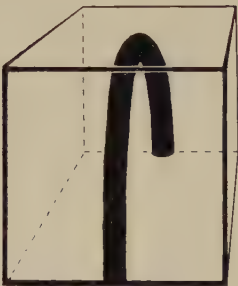
EXPLANATION OF FIGURES

8 Diagrammatic representation of the appearance of an arching strand of reticulum seen from three angles (8 a, b and c). It will be observed that when viewed from the angles of diagrams b and c the strand will assume successively the appearance of a hook and of a blunt or bulbous end. From this diagram is derived the hypothesis offered in the text that the blunt bulbous strands of reticulum actually encountered in sections (cf. figs. 9 b and c) are similar in nature.

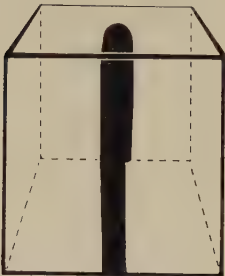
9 Subepidermal reticular strands in skin from the plantar surface of a human toe. The three locations shown (9 a, b and c) appeared within 200μ of each other in a single section and were selected to illustrate the actual appearance, in stained sections, of reticulum strands corresponding to the diagrammatic representations in figures 8 a, b and c. Rossman's fluid. Modified Pap's stain. $\times 2200$.



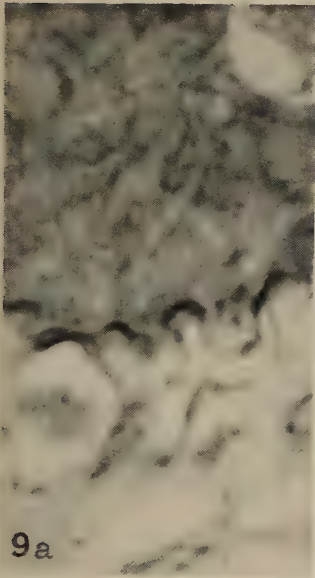
8a



8b



8c



9a



9b



9c

PLATE 3

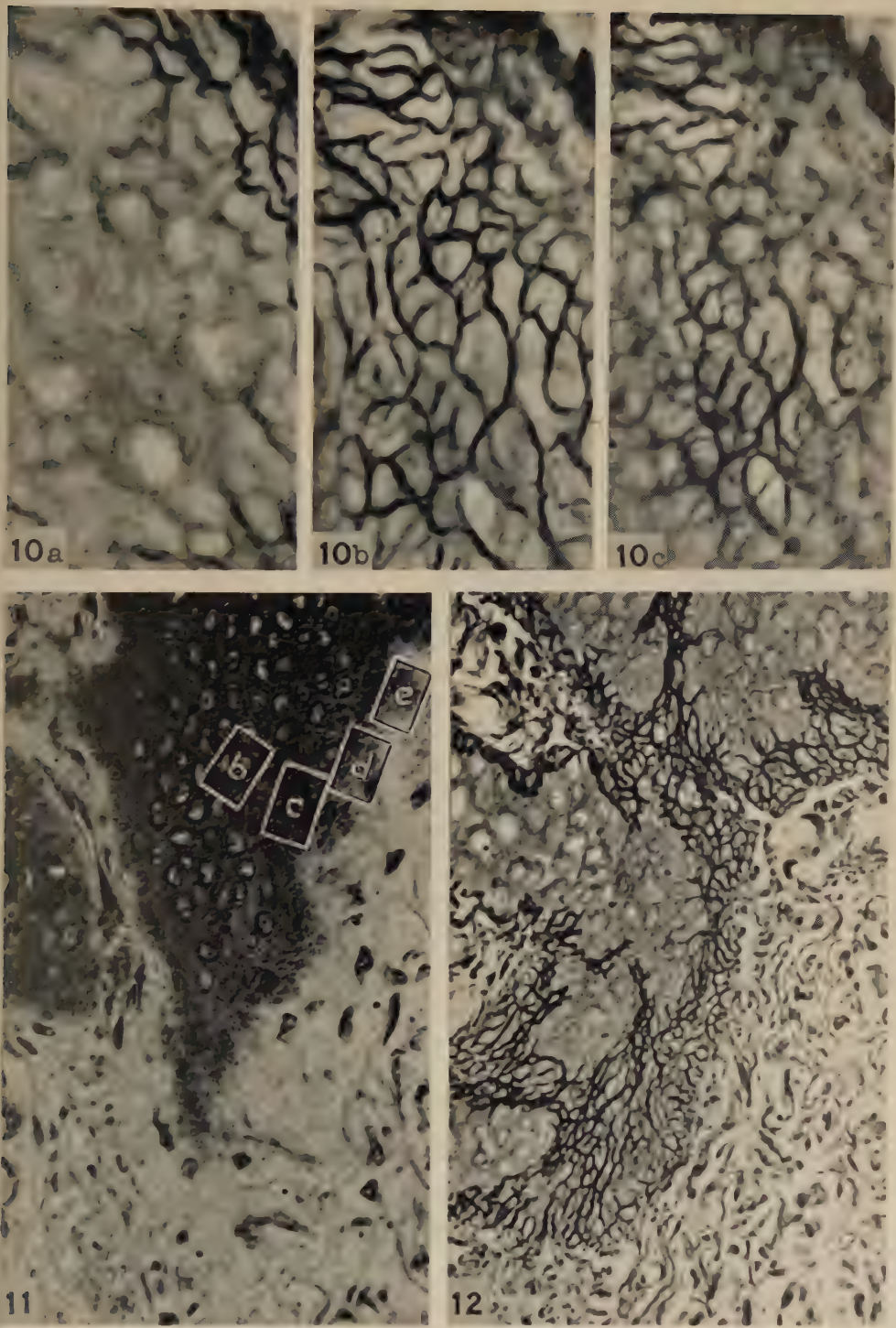
EXPLANATION OF FIGURES

10-12 Skin from the palm of a monkey sectioned parallel to the surface of the skin at levels at or near the junction between the dermis and the epidermis.

10 The appearance of the reticulum as seen in those areas of a horizontal section where the plane between the dermis and the epidermis lies nearly parallel to the skin surface. A single field is shown photographed in three successive planes of focus (10 a, b and c), from the level of the nuclei down to the sub-epidermal reticulum. Figure 10 a is focused at the level of the nuclei of the basal epidermal cells. Figure 10 b, approximately 2μ towards the dermis from 10 a, illustrates the coarse strands or bundles of dermal reticulum fibrils forming a network just below the nuclear level. Figure 10 c, situated 2μ beyond 10 b, demonstrates a more delicate, interlacing meshwork of reticular fibrils subjacent to the coarser strands seen in 10 b. These features of figures 10 b and c afford evidence that the reticular fibrils are compacted by the processes of the epidermal cells projecting into the dermis. Zenker's fluid. Modified Pap's stain. $\times 2000$.

11 A horizontal section stained with Masson's trichrome stain to demonstrate the cross-sectional appearance of the basal epidermal cells. The darkly-stained wedge-shaped area in the photograph is an epithelial ridge cut somewhat tangentially through its basal surface. The 4 rectangles labelled b, c, d and e indicate the microscopic fields from which the detailed drawings in figures 1 b, c, d and e were made. Zenker's fluid. $\times 600$.

12 A section similar to that shown in figure 11, but silvered to demonstrate reticulum. The network pattern of the reticulum, encountered frequently in horizontal sections, is evident. Significant details of the reticulum network and its relationship to the epidermis have been brought out in describing figure 10. Zenker's fluid. Modified Pap's stain. $\times 600$.



A PERSISTENT LEFT SUPERIOR VENA CAVA AND ANOMALOUS ANASTOMOSING MEDIASTINAL VEIN IN A DOMESTIC CAT¹

DONALD C. GOODMAN

*Department of Zoology, University of Illinois,
Urbana, Illinois*

TWO FIGURES

Recently in the laboratory course in Comparative Anatomy, a female cat weighing 2.9 kg and measuring 32 cm in length (atlas to sacrum) was found with the following anomalies. A persistent left superior vena cava, a closed coronary sinus, and an anastomosis between the vena cordis magna and a mediastinal vein.

Four persistent left superior vena caval systems have been found in the domestic cat. Grant ('17), Fales ('38), Caleguiri ('42), and Frontera ('50) reported the above four anomalies, all which were anatomically different. The left superior vena cava presented in this paper is most like that described by Caleguiri. Grant ('17) and Frontera ('50) reported the only two cases of closed coronary sinus in the cat and in both, a persistent left superior vena cava was present. No previous record of an anastomosis between the vena cordis magna and a mediastinal vein has been found.

The right superior vena cava, measuring 22 mm in length and 4 mm in diameter, is normally being formed by the union of the left and right innominate veins at the level of the first rib. The innominates are also normal, the left one being 16 mm and the right one 15 mm in length, and both being 3 mm

¹ Appreciation is expressed to Dr. H. M. Smith for calling attention to this specimen and for his helpful advice and to Dr. B. V. Hall for critical reading of this manuscript.

in diameter. The left superior vena cava unites with the left innominate vein 12 mm from the union of the two innominates and just medial to the junction of the jugular trunk and the subclavian vein. The left superior vena cava, measured to the right atrium, is 60 mm long and 1.3 mm in diameter. It courses caudad and 18 mm from the left innominate vein the trunk of a vein formed by two tributaries from the left dorsal

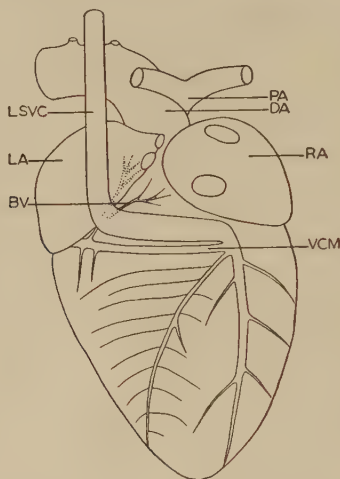


Fig. 1 Dorsal view of the heart demonstrating the persistent left superior vena cava. An, anastomosis; AZ, azygos vein; BM, mediastinal vein branches from the surface of the dorsal aorta; BT, venous branches from the thoracic wall; BV, branches from LSVC to heart; DA, dorsal aorta; EJ, external jugular vein; IJ, internal jugular vein; IM, internal mammary vein; LA, left atrium; LI, left innominate vein; LSVC, left superior vena cava; MV, mediastinal vein; PA, trunk of pulmonary artery; RA, right atrium; RI, right innominate vein; RSVC, right superior vena cava; V, vertebral vein; VCM, vena cordis magna.

thoracic wall is received. The anterior tributary collects from the third intercostal space and the posterior tributary from the fourth intercostal space; both receive tributaries from the sinus columnae vertebralis through the intervertebral canals of the third and fourth thoracic vertebrae respectively. The left superior vena cava continues caudad and enters the pericardial sac dorsal to the left atrium. At the

coronary sulcus the left superior vena cava turns to the right and follows this depression on the dorsal side of the heart to the coronary sinus ventral to the opening of the inferior vena cava into the right atrium. As the left superior vena cava turns, a small cardiac vein is received that courses obliquely, collecting over the surface of the heart between the two atria; it does not enter the right atrium. Between the entrance of this tributary and the coronary sinus, the left superior vena cava is dilated, its diameter increasing to 2 mm. The vena cordis magna, media, and parva all enter the coronary sinus normally. The coronary sinus, however, does not open into the atrium.

On the ventral side of the heart, at the point where the two cardiac veins from the surface of the interventricular septum unite to form the vena cordis magna in the coronary sulcus, an anastomosis has been formed with a mediastinal vein which collects blood from the surface of the aorta at the level of the fold of Marshall. This mediastinal vein, formed by three to four small branches from the surface of the aorta, courses craniad through the mediastinum and enters the right superior vena cava on the dorsal side at the point of its formation by the union of the left and right innominate veins. This mediastinal vein is 25 mm in length and the anastomosis is 14 mm in length giving a total length of 39 mm from the vena cordis magna to the right superior vena cava. The diameter of the mediastinal vein is normally 0.5 mm but is increased to 1 mm in this specimen (the diameter of the anastomosis being 1 mm also). The anastomosing vein passes from the vena cordis magna to the base of the pulmonary artery where it is bound in the serosa of this artery. It courses craniad, following on the ventral surface of the pulmonary arterial trunk to its division into right and left pulmonary arteries, and here the anastomosing vein passes through the heavy serosa of the fold of Marshall, ventral to a prominent ligamentum arteriosum and across the ventral surface of the aorta to join the mediastinal vein at the point where all of the branches from the surface of the

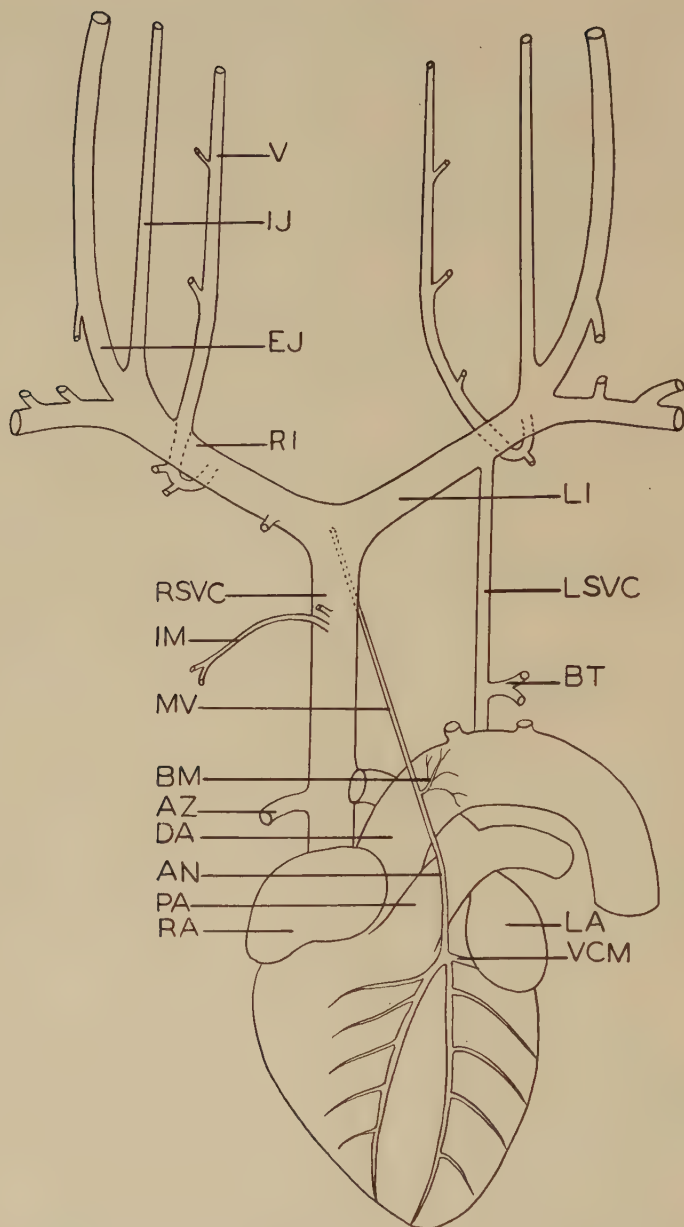


Fig. 2 Ventral view of heart and thoracic veins demonstrating the anastomosing vein and persistent left superior vena cava.

aorta unite to form the main trunk of this mediastinal vein. The fact that the anastomosing vein is fully injected suggests that it was a functional connection. The other anomalous veins are also fully injected and it is likely that they too were functional.

Normally in the adult cat, all that is left of the left superior vena cava is the coronary sinus. The left superior vena cava in this specimen was formed by the retention of the left pre-cardinal vein and the duct of Cuvier. The branch from the left superior vena cava to the left third and fourth intercostal spaces is a retention of the left anterior part of the supracardinal. In the embryo, the supracardinals collect blood in the thoracic region chiefly from the body wall. This blood is returned to the heart through the right and left ducts of Cuvier in common with the blood collected from the head region and anterior limbs by the precardinals. In the normal adult cat these intercostal spaces are drained into the azygos vein.

Hutton ('15) gives two explanations for the separation of the coronary sinus from the right atrium. The first explanation suggests that a composite structure is formed, being the result of a fusion between the coronary segments of the right and left venous valves. The second explanation is that an unusually voluminous Thebesian valve fuses with the margins of the opening of the coronary sinus. A detailed discussion of this found in Hutton's paper.

The anastomosis between the vena cordis magna and a mediastinal vein described above has no apparent embryological significance similar to that of the left superior vena cava and the left supracardinal, but is rather a compensatory mechanism enabling the blood of the cardiac veins to be returned to the right atrium. The mediastinal vein retains its function of draining the surface of the aorta and in addition carries blood from the cardiac veins to the right superior vena cava.

SUMMARY

The unusual anomalies of a persistent left superior vena cava, a closed coronary sinus, and an anastomosis between the vena cordis magna and a mediastinal vein found in a female cat are described. An explanation for the presence of this anastomosis is proposed.

LITERATURE CITED

- CALEGUIRI, J. V. 1942 Notes on the persistence of the left precardinal vein in the adult cat. *Proc. West Virg. Acad. Sci.*, 75: 77-78.
- CHOUKE, K. S. 1939 A case of bilateral superior vena cava in an adult. *Anat. Rec.*, 74: 151-157.
- EALES, N. B. 1938 Paired precaval veins in a kitten. *Anat. Anz.*, 86: 68-71.
- FRONTERA, J. G. 1950 Anomalous persistent left anterior cardinal system draining the coronary blood in a domestic cat. *Anat. Rec.*, 106: 127-130.
- GRANT, S. B. 1917 A persistent superior vena cava sinistra in the cat transmitting coronary blood. *Ant. Rec.*, 13: 45-49.
- HUTTON, W. K. 1915 An anomalous coronary sinus. *J. of Anat. and Phys.*, 49: 407-413.
- STOLAND, O. O., AND LATIMER, H. B. 1947 Persistent left superior vena cava in the dog. *Trans. Kansas Acad. Sci.*, 50: 84-86.

A HISTOLOGICAL STUDY OF THE ALIMENTARY CANAL OF THE COTTON RAT, SIGMODON HISPIDUS

ORVILLE E. BLANK

Department of Biology, University of Florida, Gainesville

SIXTEEN FIGURES

INTRODUCTION

The cotton rat, *Sigmodon hispidus*, has become an important laboratory animal since Brigham ('37), reported the cotton rat to be susceptible to endemic typhus fever and Armstrong ('39), demonstrated the presence of the poliomyelitis virus in the animal. My inability to find published information on the histology of the alimentary canal of this animal led to the investigation reported here.

Each part of the alimentary canal described in this paper is based on a study of materials from 3 to 7 animals. Cross and longitudinal sections, stained with general and selective stains, were utilized. In all, 20 animals were used in the investigation. All measurements here recorded were taken of mature specimens whose body weights were between 90 and 110 gm. All animals were fed a normal laboratory diet previous to removal of the alimentary canal.

The procedure used in preparing materials for study was as follows: each animal was anaesthetized with ethyl ether, and while in this condition, the coelom was injected with the fixative under a pressure of 1.4 to 1.7 pounds per square inch for a period of 4 to 10 hours. By this method, the entire alimentary canal was fixed except for the cranial end of the esophagus and the most caudal portion of the rectum. The fixatives and stains used, unless otherwise mentioned, are

according to Conn and Darrow ('46). They are as follows: 1:10 formalin, formalin-aceto-alcohol (FAA), and picro-formalin (Bouin's). Dioxane was used as the dehydrating and clearing agent. The stains were standard alum-haematoxylin, triosin, eosin, Foot's ammoniated silver carbonate, Van Gieson's stain with alum-haematoxylin and modified Bodian's protargol stain. Measurements of the different regions of the alimentary canal are given in table 1.

TABLE 1

	TUNICA MUCOSA			TELA SUBMUCOSA	TUNICA MUSCULARIS	
	E.P.	L.P.	L.M.M.		Inner	Outer
Oesophagus						
Upper	50	10-13		10-28	105-145	72-88
Lower	55	10-13	8-13	10-28	105-145	72-88
Stomach						
Oesophageal	30-90	7-21	14	8-20	70	35
Cardiac	8-11	2-3	7-10	28	91-105	140-168
Fundic	8-11	2-3	7-10	28	33	7
Pyloric	8-11	2-3	13-59	21-28	88-91	7
Pyloric sphincter	8-11	¹	¹	420	126-133	10-13
				Ant. 420		
Duodenum	11	20-30	0-4	10-24	10-14	5-7
Jejunum	8-11	15-25	1-3	5-13	26-42	13-18
Ileum	8-11	20-25	1-3	7-28	24-28	7-8
Caecum	8-11	¹	1-3	14-280	70-105	21-70
Colon	8-11	84-156	11-15	3-10	23-28	7-8
Upper	+	+			Inter sphic. 112	
Rectum	20	2	0	140		
Lower	—	—			Exter sphic. 126	

¹ Measurements for these regions cannot be definitely made.

The tunica serosa is 7 to 10 μ in thickness except in the oesophagus and rectum.

ESOPHAGUS

Maximow and Bloom ('46), state that in mammals that consume coarse vegetable foods (rodents, ruminants, and horse) the stratified squamous epithelium of the esophagus undergoes cornification. In *Sigmodon*, the epithelium is

cornified, the innermost layer being scaly or horny. The cells of the basal layer of the epithelium are columnar (fig. 2).

The lamina propria is composed of moderately loose collagenous tissue bound together by reticular fibers which are few in number. The latter were readily demonstrated by Foot's ammoniated silver carbonate method.

The lamina muscularis mucosae is composed of smooth muscle and is absent at the extreme cranial end of the esophagus, first appearing approximately one-quarter of the way down the esophagus as isolated bundles of sparsely distributed smooth muscle. The muscularis mucosae in the esophagus reaches its highest development in the caudal region. Here the bundles of muscle cells become sufficiently numerous to form a thin but complete band of longitudinally disposed smooth muscle.

The longitudinal folds in the esophagus involve the epithelium, lamina propria, lamina muscularis mucosae and part of the tela submucosa (fig. 2). There are neither cardiac nor esophageal glands present in the esophagus.

The tunica muscularis is thickest at the caudal end of the esophagus. This layer is remarkable in that it is composed of striated muscle throughout the entire length of the esophagus. Bremer ('46), states that this condition is found in rabbits. In the caudal region of the esophagus of *Sigmodon*, there are several variations in the arrangement of the muscle fibers of the outer longitudinal layer of the tunica muscularis. In some specimens, the fibers adjacent to the circular fibers are not longitudinal, but are spiralled around the esophagus. In other specimens, there are two bands of longitudinal muscle fibers separated by a band of circular muscle, 1 to 4 fibers thick (fig. 4). A less common variation is where two or three muscle fibers arise in the inner circular layer and pass through the areolar connective tissue layer to intermingle with the fibers of the outer longitudinal layer (fig. 3).

STOMACH

The stomach in *Sigmodon* is composed of 4 distinct regions as was found by Kammeraad ('42), and Sharples ('45), who investigated the "rat." These areas are shown in figure 1. The epithelium is of two types; stratified squamous in the esophageal portion and simple columnar in the glandular portion. According to Toepfer (1891), in his histogenic work on *Mus musculus*, the epithelium arises as simple cuboidal epi-

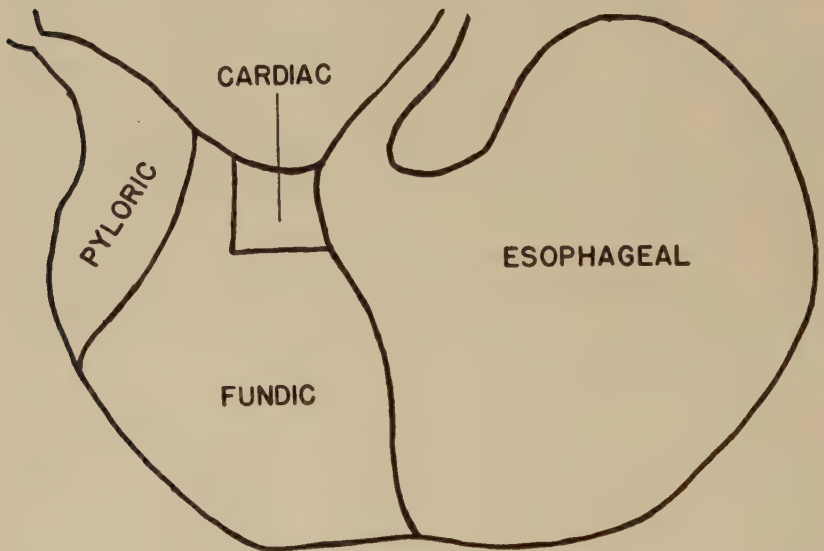


Fig. 1 Distribution of the 4 regions of the stomach of the cotton rat, *Sigmodon hispidus*.

thelium and later becomes stratified. Kammeraad ('42) states that the epithelium of the esophageal portion of the "rat" stomach becomes stratified when the embryo is 18 days old. Cornification begins on the 19th day.

Esophageal stomach. The esophageal stomach resembles the esophagus insofar as the structure of the tunica mucosa is concerned. The epithelium is cornified like that of the esophagus. Small folds of the tunica mucosa extend into the lumen. The lamina propria extends into these folds. There

are no gastric glands or argentaffin cells in this region of the stomach. The tunica muscularis is composed of smooth muscle.

Transition from esophageal to cardiac stomach. The cardiac region and a small portion of the esophageal region lie caudal to the entrance of the esophagus into the stomach. Part of the tunica muscularis that forms the esophageal sphincter extends into these two regions. At the junction of the esophageal and cardiac regions, the tunica mucosa of the esophageal portion forms a large fold projecting into the lumen of the stomach. The bulk of this fold is composed of lamina propria. The lamina muscularis mucosae does not extend deeply into this fold. There is an abrupt change from stratified squamous to simple columnar epithelium at the junction of the two regions.

Cardiac stomach. The cardiac stomach in the cotton rat is extremely small. Parietal cells are very abundant in this region of the stomach (fig. 5). It has the appearance of a transitional area between the cardiac and fundic regions of the stomach. It forms a saddle-like band over the lesser curvature of the stomach. It extends approximately $690\ \mu$ from its junction with the esophageal portion of the stomach. The extension of the cardiac region on either side of the stomach was not exactly determined by serial sections of the entire region, but from the material observed it is estimated that the total width ranges from one to one and one-half millimeters.

The bulk of the tunica mucosa is modified into cardiac glands. These glands are usually of the simple, tubular variety, the basal end of which is coiled, but show evidence of branching. The gastric pits or foveolae gastricae are 78 to $89\ \mu$ deep. The cells lining the foveolae gastricae are the non-secretory, columnar type. The secretory portion of the glands is 161 to $182\ \mu$ deep and is composed of parietal, mucous and argentaffin cells.

The argentaffin cells are very few in number. Dawson ('45, '48), working on the distribution of argentaffin cells in the

“rat,” rabbit, guinea pig, mouse, and hamster, states that these cells are less abundant in the cardiac than in any other glandular region of the stomach. Argentaffin cells are approximately one-half the size of the parietal cells, and are irregular in shape. The best preparations for demonstrating argentaffin cells were obtained by using Bodian’s protargol stain (Dawson, ’44).

The mucous cells are generally cuboidal in shape and approximately one-half the size of the parietal cells.

The lamina propria in the glandular portion of the stomach is thin and projects between the gastric glands. It is composed of collagenous fibers which are bound together by fine reticular fibers. The collagenous fibers are thin and usually do not exceed 3 or 4 in number between the individual glands. The lamina muscularis mucosae is similar to that of the esophagus.

Transition from cardiac to fundic stomach. The transitional area between the cardiac and fundic stomach is narrow. The first noticeable change toward the fundic condition is an increase in the number of parietal cells and a decrease in the number of mucous cells. Near the fundus, the mucous cells disappear and are replaced by chief cells. The depth of the foveolae gastricae begins to increase in the area where the first chief cells appear, quickly attaining a depth of between 49 to 63 μ . There is a gradual decrease in the thickness of the tunica muscularis from approximately 250 μ in the cardiac region to approximately 46 μ in the fundic region.

Transition from esophageal to fundic stomach. The transition from the esophageal to fundic stomach is very abrupt as was true in the transition from the esophageal to cardiac stomach. The outstanding difference between these two transitional areas is the thickness of the tunica muscularis which is thin in the esophageal-fundic transition and thick in the esophageal-cardiac transition.

Fundic stomach. The fundic region is the largest of the three glandular portions of the stomach. The fundus forms a band extending completely around the stomach; narrow on

the side of the lesser curvature and broad on the side of the greater curvature.

The epithelium of the fundus is very similar to the epithelium in the cardiac stomach. The bulk of the tunica mucosa is involved in the formation of the fundic glands. They are of the branched tubular type; the branching occurs in the basal quarter. The foveolae gastricae are approximately one-fifth of the depth of the entire gland. The secretory portion of the glands is 160 to 170 μ in depth. Four types of cells are found in the fundic glands, which, in descending order of abundance, are parietal, chief, mucous neck and argentaffin. Although the argentaffin cells are few in number, they are still more numerous here than in any other part of the stomach (fig. 8). They are most abundant in the basal end of the glands. The parietal cells occur throughout the secretory portion of the glands but are most abundant in the superficial third. The chief cells are present in the basal two-thirds of the secretory portion but are most abundant at the bases of the glands (fig. 6).

Transition from fundic to pyloric stomach. The transition from fundic to pyloric stomach is not abrupt, and is marked by gradual changes in the tunica mucosa and tunica muscularis. The tunica mucosa decreases in thickness from 210–273 μ in the fundic to 161–210 μ in the pyloric region; the foveolae gastricae increase in depth from 49–63 μ in the fundic region to 91–105 μ in the pyloric region. The foveolae gastricae in the fundus are approximately one-fifth the depth of the entire gland as compared to approximately one-half the depth in the pyloric region. The chief cells become few in number in the middle portion of the transitional area and disappear completely in the caudal region. In the transitional area near the fundus, the inner circular layer of the tunica muscularis is 33 μ and the outer longitudinal layer is 7 μ .

Pyloric stomach. The pyloric region is small. The secretory portion of the glands measures from 98 to 140 μ in depth. The glands are of the simple, branched tubular type. These glands contain only one type of cell which are cuboidal in

shape with the nuclei located at the bases of the cells. In sections stained with haematoxylin and triosin, they resemble cells found in Brunner's glands.

Transition from pyloric stomach to duodenum. The transition to duodenum is very abrupt so that there is little or no transitional area (fig. 7). Brunner's glands, which are characteristic of the duodenum, are the only glands in the alimentary canal of *Sigmodon hispidus* that extend into the tela submucosa. The transitional area is characterized by an abrupt thickening of the tunica muscularis, and the complete separation between the circular muscle layer of the pylorus and the duodenum.

The tela submucosa in the pyloric end of the duodenum is extremely thick, but thin in other regions. The inner circular layer of the tunica muscularis in the pyloric sphincter is broken into numerous bundles by connective tissue septa. The longitudinal layer in the pyloric stomach is continuous with the longitudinal layer in the duodenum.

Duodenum. Caudal to the pyloric sphincter, the duodenum bends through approximately a 45° angle to the right and then extends caudally for an average of 3 to 4 cm. The diameter of the lumen is large in relation to the remainder of the small intestine.

The villi of the duodenum are 126 to 140 μ in length (fig. 9). The epithelium of the villi is composed of tall columnar cells with striated borders and occasional goblet cells. The columnar cells lose their striated borders near the openings of the crypts of Lieberkühn. The lamina propria of the duodenum contains diffuse lymphoid tissue and thereby differs from the lamina propria of the esophagus and stomach. In many areas of the tunica mucosa, the lymphatic tissue is dense, forming solitary ovoid nodules. In the region where Brunner's glands are present, the lamina muscularis mucosae is broken because these glands extend through it into the tela submucosa. Where Brunner's glands are lacking, the lamina muscularis mucosae lies along the bases of the glands of Lieberkühn.

Brunner's glands are extremely abundant near the junction of the pylorus and duodenum. The terminal portions of Brunner's glands are highly branched and coiled and are invested in fibrous connective tissue. The secretory portions of these glands are composed of low columnar cells; their nuclei are small and are located at the basal ends of the cells. The lumen of these glands ranges from 7 to 13 μ in diameter. The epithelium of the glands of Lieberkühn is composed of low columnar and goblet cells. Paneth cells are generally found in the basal ends of the glands. Argentaffin cells are more numerous in the duodenum than in the fundic stomach and are found between the cells lining the glands.

Jejunum. The structure of the jejunum is essentially the same as that of the duodenum in regard to the epithelium of the villi and the cells of the glands of Lieberkühn. Few argentaffin cells are present in the jejunum. The villi of the jejunum are longer than those of the duodenum and ileum and measure 314 to 502 μ in length and 34 to 42 μ in diameter.

Ileum. The general structure of the villi in the ileum is the same as that of the villi in the duodenum and jejunum. The glands of Lieberkühn are similar to those discussed above. There are fewer argentaffin cells in the ileum than any other region of the small intestine. The villi are 147 to 199 μ long and have a diameter of 40 to 55 μ .

Ileocaecal valve. It is formed by the projecting of the tunica mucosa of the ileum into the lumen of the caecum and is supported by the tunica muscularis of the ileum (fig. 10).

The abrupt transition from the tunica mucosa of the ileum to that of the caecum occurs on the valve. There is no appreciable difference in the thickness of the tunica mucosa of the ileum and the caecum, but villi are present in the former and lacking in the latter. The lamina muscularis mucosae is somewhat thickened in the region of the ileocaecal valve.

The tela submucosa begins to thicken in the region of the valve, and attains an average thickness of 56 μ . The valve is strengthened at its base by the overlapping of the circular

layers of the tunica muscularis of both the ileum and the caecum. As noted above, some of the smooth muscle cells of the inner circular layer of the ileum extend into the ileo-caecal valve. There is very little overlapping of the outer longitudinal layer of muscle. The inner circular layer of tunica muscularis is $168\ \mu$ thick where overlapping occurs. The outer layer of muscle is only 7 to $9\ \mu$ in thickness.

Caecum. The walls of the caecum are thin. The tunica mucosa is raised into folds that extend into the lumen (fig. 11). The ileum enters approximately the middle of the caecum.

The surface epithelium is composed of simple columnar cells with striated borders. The epithelium of the ducts of the caecal glands, which are of the simple tubular type, consists of columnar and goblet cells. Argentaffin cells are more abundant here than the jejunum or ileum. The bulk of the tunica mucosa is modified into these glands and the lamina propria lies between them. The glands in the caecum of the cotton rat do not resemble the glands of Lieberkühn in the small intestine or colon. After fixation and staining with hematein in an aqueous solution, the cells in these glands possess a pale, irregular cytoplasmic network with large, empty meshes and a flattened dark nucleus at the base, and thus resemble mucous cells. The tunica mucosa varies from 140 to $350\ \mu$ in thickness. The lamina muscularis mucosae is thin and is usually not more than two or three cells in thickness.

Transition from caecum to colon. The folds of the tunica mucosa in the caecum become more extensive and numerous in the transitional region between the caecum and the colon (fig. 12). These folds contain large numbers of simple tubular mucous glands. The cytoplasm of the secretory cells in the caecal glands is more abundant and translucent than that in the cells of the glands of the colon. The caecum and colon can be separated histologically on the basis of this cytoplasmic difference. Glands are quite numerous in the colon near the region of the caecum, but become less numerous caudal to the caecum.

Colon. The surface epithelium between the openings of the glands of Lieberkühn is composed of columnar cells with striated cuticular borders. The glands of the colon are of the simple tubular type and they extend from the free surface almost to the lamina muscularis mucosae (fig. 13). The necks of the glands are composed of columnar and numerous goblet cells. No Paneth cells were observed and very few argentaffin cells. The lamina propria in the colon contains a greater amount of diffuse lymphoid tissue than in the small intestine. There are also solitary nodules of lymphoid tissue present. The lamina muscularis mucosae is highly developed in this section of the tract.

Rectum. In general appearance the upper rectum resembles the colon, but the glands, while similar to those in the colon, are not as numerous nor as deep. The lower rectum is further divided into three regions; the zona columnaris, zona intermedia, and zona cutanea.

Upper rectum. The epithelium of the upper rectum is simple columnar. The tunica mucosa is 154 to 182 μ thick and contains the rectal glands which appear to be mucous glands. The tela submucosa is approximately 234 μ in thickness. The inner circular layer of the tunica muscularis is 140 to 154 μ in thickness. The outer longitudinal layer is 42 to 49 μ thick.

Lower rectum. Zona columnaris. The epithelium is simple columnar (fig. 14). The tunica mucosa contains mucous glands. The zona columnaris ends where the stratified squamous epithelium of the zona intermedia begins. The tela submucosa resembles the submucosa of the upper rectum and is about 112 μ in thickness. The inner circular layer of the tunica muscularis is smooth muscle and forms the internal anal sphincter. The outer longitudinal muscle is almost lacking in this region.

Zona intermedia. The zona intermedia begins cranial where the first stratified squamous epithelium appears and ends caudally where the first hair follicles appear (figs. 14, 15). The epithelium is cornified except for its most cranial portion. The tunica mucosa is 24 to 28 μ in thickness. No mucous

glands are present in this region of the rectum. The lamina propria and lamina muscularis mucosae are almost completely lacking. The inner circular muscle layer is striated and forms the external anal sphincter. It is 126 to 140 μ in thickness. The outer longitudinal layer of muscle is completely lacking except for a few isolated patches.

Zona cutanea. The zona cutanea is formed by the skin immediately surrounding the anus (fig. 15). The epithelium is of the cornified, stratified squamous type. There are a large number of hair follicles and sebaceous glands present. The 4 layers characteristic of the alimentary canal are lacking in the zona cutanea. In this region the epithelium is attached by connective tissue directly to the underlying skeletal muscle. No anal glands were found.

SUMMARY

Stratified squamous epithelium is present in the esophagus and esophageal stomach, and from the zona intermedia through the zona cutanea of the rectum. The epithelium is cornified in the above regions except for the region of the zona intermedia adjacent to the zona columnaris. The epithelium in the remainder of the alimentary canal is simple columnar. The columnar cells in the small intestine, caecum, and colon have pronounced striated borders. The columnar cells in the openings of the glands do not have striated borders. The lamina muscularis mucosae is absent in the most cranial region of the esophagus and in the zona cutanea of the rectum.

No glands are present in the esophagus or esophageal stomach. The glands in the cardiac stomach of the cotton rat differ from those found in man in that they contain an extreme abundance of parietal cells. Argentaffin cells are more abundant in the fundic stomach than in any other region of the stomach. However, they are present in all three glandular portions of the stomach.

Glands of Lieberkühn are present in the small intestine, caecum, and colon. Except for those in the caecum, the above

glands are lined with low columnar epithelium and goblet cells. Paneth cells are present in the basal region of the glands. The cytoplasm of the secretory cells in the caecal glands is more abundant and translucent than that in the cells of the glands of the colon. The caecum and colon can be separated histologically on the basis of this cytoplasmic difference. The cells in the caecal glands appear to be mucous cells.

Argentaffin cells are slightly more abundant in the duodenum than in any other region of the alimentary canal. They decrease in number in the small intestine toward the caecum. Argentaffin cells are relatively numerous in the caecum but are almost lacking in the colon. No anal glands were found.

The tunica muscularis is variable both in structure and thickness. Striated muscle is present throughout the entire length of the esophagus and lower rectum. In the remainder of the canal, the tunica muscularis is composed of smooth muscle. Variations from the longitudinal and circular arrangement of the muscle fibers are not uncommon in the esophagus. There is a complete separation between the circular layer of the tunica muscularis in the pyloric stomach and the duodenum. On the other hand, the longitudinal layer of muscle in the pylorus is continuous with that of the duodenum.

LITERATURE CITED

- ARMSTRONG, CHARLES 1939 The experimental transmission of poliomyelitis to the eastern cotton rat, *Sigmodon hispidus hispidus*. Public Health Reports, Washington, D. C., 54: 1719-1721.
- BREMER, J. LEWIS 1946 A textbook of histology, 6th ed. Blakiston, Philadelphia. 1-723.
- BRIGHAM, GEORGE D. 1937 Susceptibility of animals to endemic typhus fever. Public Health Reports, Washington, D. C., 52: 660-662.
- CONN, H. J., AND MARY A. DARROW 1946 Staining procedures used by the Biological Stain Commission. Geneva, N. Y.
- DAWSON, A. B. 1944 Bodian's protargol method applied to other than neurological preparations. St. Tech., 19: 115-118.
- 1945 Argentaffin cells of the gastric mucosa of the rabbit, guinea pig, mouse and hamster. Anat. Rec., 91: 53-63.
- 1948 Argentophile and argentaffin cells in the gastric mucosa of the rat. Anat. Rec., 100: 319-329.

- KAMMERAAD, ADRIAN 1942 The development of the gastro-intestinal tract of the rat. I. Histogenesis of the epithelium of the stomach, small intestine, and pancreas. *J. Morph.*, 70: 323-351.
- MAXIMOW, ALEXANDER A., AND WILLIAM BLOOM 1946 A textbook of histology, 4th ed. W. B. Saunders Co., Philadelphia, 1-833.
- SHARPLES, WYNNE 1945 The histogenesis of argentaffin cells in the stomach and duodenum of the rat. *Anat. Rec.*, 91: 107-117.
- TOEPFER, K. 1891 Die Morphologie des Magens der Rodentia. *Morph. Jahrbuch.*, Bd. 17, S. 380-407.

PLATE 1

EXPLANATION OF FIGURES

2 Cross section of the esophagus mid-way between the pharynx and the stomach showing the cornification of the stratified squamous epithelium, stratum germinativum, lamina propria, lamina muscularis mucosae, and tela submucosa. Stained with standard alum-haematoxylin and triosin. $\times 470$.

3 Cross section of the esophagus mid-way between the pharynx and stomach showing variation in the arrangement of the fibers of the tunica muscularis which is striated muscle. Alum-haematoxylin and triosin. $\times 350$.

4 Cross section of the esophagus mid-way between the pharynx and stomach showing variation in the arrangement of the fibers of the tunica muscularis. Alum-haematoxylin and triosin. $\times 350$.

5 Longitudinal section through the basal one-third of the cardiac glands showing both parietal and secretory cells of the cardiac glands. Alum-haematoxylin and triosin. $\times 625$.

6 Longitudinal section of the basal quarter of the fundic glands showing chief and parietal cells. Alum-haematoxylin and triosin. $\times 600$.

7 Longitudinal section through the pyloric sphincter showing Brunner's glands, glands of Lieberkühn, villi of the duodenum, and a solitary nodule of lymphatic tissue. Alum-haematoxylin and triosin. $\times 100$.

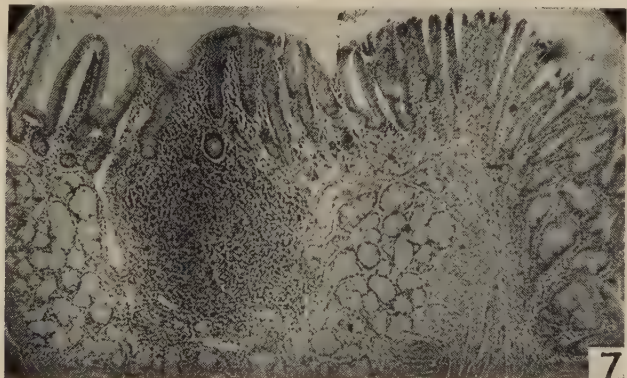
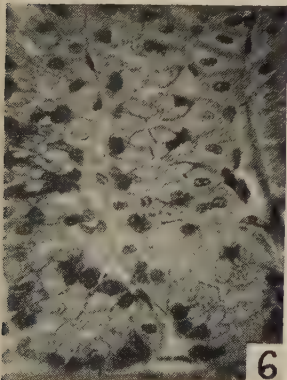
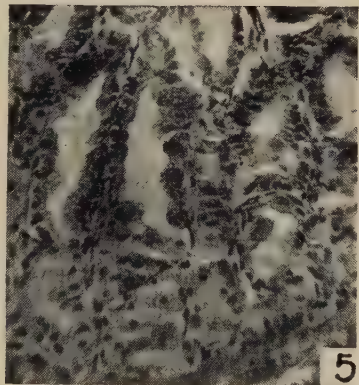
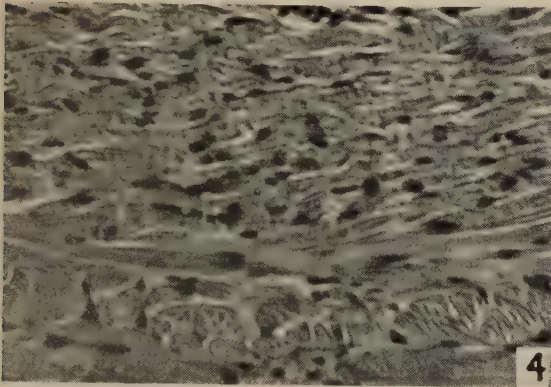
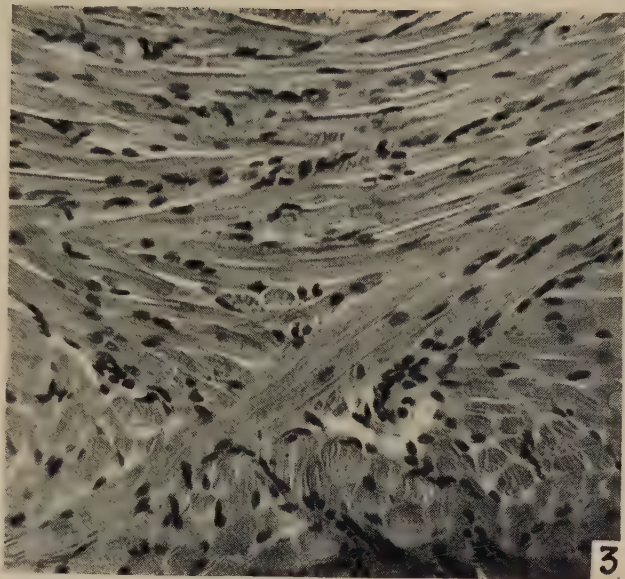
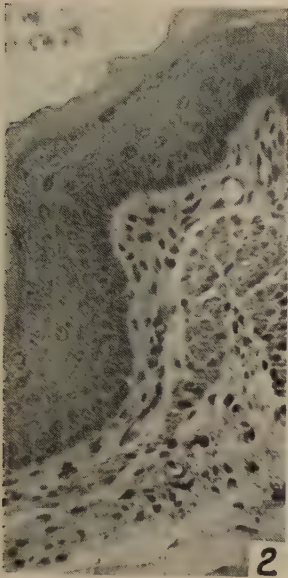


PLATE 2

EXPLANATION OF FIGURES

8 Cross section of the proximal quarter of the fundic glands showing argen-taffin cells. Prepared by the modified Bodian method. $\times 600$.

9 Cross section through the duodenum showing villi and glands of Lieberkühn. Alum-haematoxylin and triosin. $\times 135$.

10 Longitudinal section through the ileocaecal valve showing the valve projecting into the lumen of the caecum and the inner circular layer of the tunica muscularis of the ileum extending into the ileocaecal valve. Alum-haematoxylin and triosin. $\times 130$.

11 Longitudinal section through the caecum showing folds in the tunica mucosa and caecal glands. Alum-haematoxylin and triosin. $\times 110$.

12 Longitudinal section through the transitional area between the caecum and the colon. The caecum is on the left side of the figure. Alum-haematoxylin and triosin. $\times 115$.

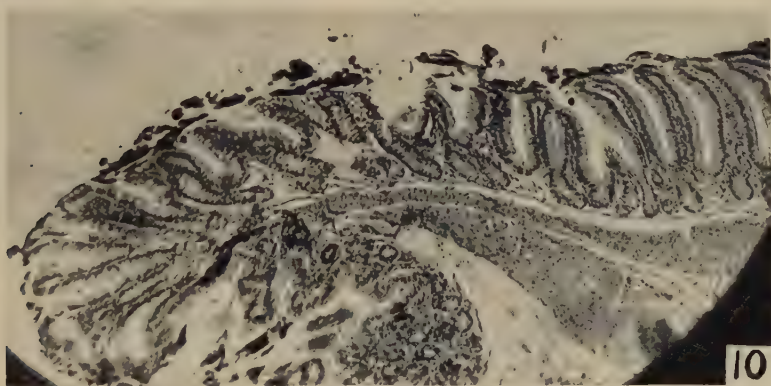
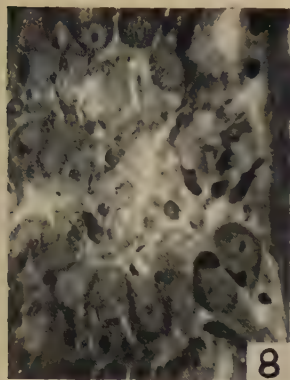
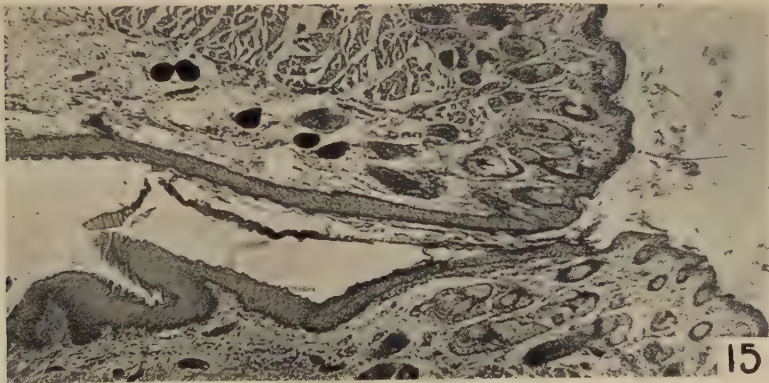
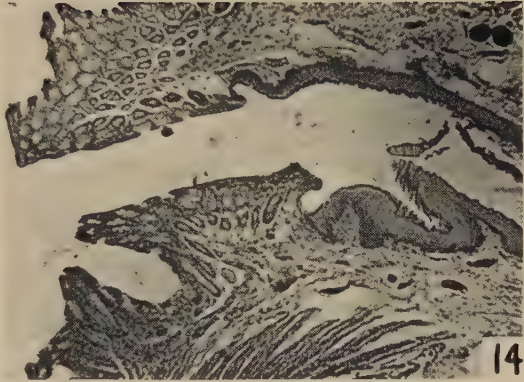
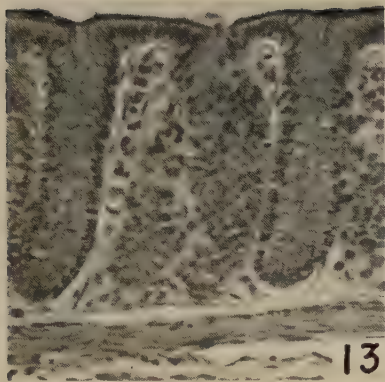


PLATE 3

EXPLANATION OF FIGURES

- 13 Cross section through the tunica mucosa of the colon. Alum-haematoxylin and triosin. $\times 460$.
- 14 Longitudinal section of the lower rectum showing the zona columnaris and zona intermedia. Alum-haematoxylin and triosin. $\times 100$.
- 15 Longitudinal section of the lower rectum including the anus and showing the zona intermedia and zona cutanea. $\times 110$.



ESTROGENIC ACTIVITY OF ADRENAL TRANSPLANTS TO THE UTERUS OF OVARIECTOMIZED RATS

MARVIN J. WEINSTEIN, JOSEPH SCHILLER AND
HARRY A. CHARIPPER

*Departments of Biology, New York University and Long Island University,
New York, N. Y.*

ELEVEN FIGURES

INTRODUCTION

Considerable evidence has been accumulated to indicate an inter-relationship between the adrenal cortex and the gonads (Parkes, '45). The participation of the adrenal cortex in the estrous cycle has been confirmed under various natural and experimental conditions. The administration of adrenocorticotrophic hormone has been shown to produce estrogenic responses in immature ovariectomized rats (Moon, '37; Nelson, '41), partially hepatectomized female rats (Schiller, '45) and in ovariectomized rhesus monkeys (Schiller, '46). Corey and Britton ('34) restored the estrous cycle in adrenalectomized rats by the injection of cortico-adrenal extract. The occurrence of artificial estrous cycles in ovariectomized rats by injections of constant threshold doses of estrone has been related to a specific and direct influence of the adrenal gland (Bourne and Zuckerman, '41). The evidence presented by these investigators is not considered as conclusive proof that the adrenal cortex influences the estrous cycle directly through the release of sex hormones rather than hormones which act on the general metabolism.

The absence of any effect of adrenal cortical secretions on the accessory reproductive structures of ovariectomized animals suggested the idea that the dilution of the hormone in

the total blood volume lowers its concentration to a point below which it cannot elicit a response. The purpose of this investigation is to remove this dilution factor by utilizing the technique of transplantation of the adrenal cortex to the site of the target organ, thus permitting the cortical secretions to penetrate directly into the genital tract. The present work, therefore, is concerned with the effects of autografting adrenal tissue to the uterus of ovariectomized rats.

MATERIALS AND METHODS

Adult female albino rats of the Wistar strain ranging in weight from 140 to 200 gm were employed in these experiments. A total of 18 animals were used. All animals were maintained under standard laboratory conditions and fed Purina laboratory chow supplemented with occasional bread and lettuce. Vaginal smears by the lavage method were made of all animals for 10 days before undergoing operation. Only those animals exhibiting a normal estrous cycle were utilized. All operations were performed under ether anesthesia. The 12 animals comprising the experimental group were maintained on a 1% solution of sodium chloride given as drinking water for approximately 30 days, at which time tap water was substituted for a minimum of an additional 30 days. Vaginal smears were made of all animals daily until the end of the experiment. Animals were sacrificed at intervals of from 57 to 107 days after transplantation.

The experimental animals were divided into two groups. Both groups were ovariectomized and adrenalectomized, and in one group of 6 animals both adrenals were autografted to the wall of the uterus above its junction with the vagina; and in the second group of 6 animals, a portion of the adrenal capsule was placed in a pocket in the same area of the uterus. The control group of 6 animals was ovariectomized by the dorsolateral approach and the adrenal glands were left in situ.

Adrenalectomy and ovariectomy in the experimental group was performed in one step through an incision made in the

mid-ventral line. It was determined that a single incision by the ventral approach would limit the time of operation and decrease the duration of anesthesia. The ovary and its surrounding fat was excised together with a portion of the oviduct. A hemostat was left in place to facilitate stasis while the second ovary was removed in the same fashion. The adrenal glands and their surrounding fatty tissue were excised and placed immediately in cotton soaked in warm physiological saline. Both adrenal glands were autografted to the wall of the uterus above its junction with the vagina. The incision was closed and coated with collodion to facilitate healing and to discourage the animal from opening the wound.

The adrenals and ovaries were removed in the same fashion in the experimental group in which a portion of the adrenal was implanted in a pocket in the wall of the uterus. After cleaning one adrenal of surrounding tissue, the gland was enucleated and approximately one-sixth of the capsule was placed in a pocket in the muscularis of the uterus made with an iridectomy scalpel, being careful not to penetrate to the lumen. The lips of the pocket were closed over the portion of the adrenal capsule using fine silk thread lightly tied. The incision was closed in the same manner as in the first group of animals.

At the conclusion of the experiment the animals were placed under nembutal anesthesia and the reproductive accessories observed macroscopically. A careful search was made to ascertain whether any ovarian or accessory adrenal regeneration had taken place. The uterus and vagina were excised in toto and the animal was then sacrificed by ex-sanguination. The uterus and vagina were placed in Bouin's picro-formol fixative, serially sectioned in paraffin at 10μ and stained with Harris' hematoxylin and eosin.

EXPERIMENTAL RESULTS

Adrenal transplantations proved successful in all animals and autopsy failed to disclose any ovarian or accessory cortical regeneration. While vaginal smears show a constant

diestrus phase, macroscopically, however, the reproductive tract does not have the appearance expected in a typical castrate condition. The uterus and vagina are consistently larger in the experimental animals than in the controls. There is a high degree of vascularity in the area of the transplanted adrenal and in the entire genital tract.

Adrenal. Histological examination of the adrenal transplants discloses that in the experimental group with the entire adrenal glands sutured on the wall of the uterus, all three zones of the cortex are present (figs. 1, 4). There is no histological evidence of the adrenal medulla having been sustained. The zona fasciculata appears to be larger than in the normal gland.

In the experimental group with a portion of the capsule implanted in a pocket in the wall of the uterus, regeneration of the zona reticularis is not evident in any case, and numerous blood sinuses are present (fig. 2). In three cases, the zona glomerulosa and zona fasciculata were present and in three cases only zona glomerulosa type of cells were present, although covering a greater area than in the normal gland.

Uterus. The stroma and myometrium, although narrow and atrophic, are more edematous than in the castrate type (figs. 2, 3). The uterine mucosa is generally of the typical castrate type (fig. 4). With only two exceptions, the epithelial cells are low cuboidal, almost squamous in appearance. In these two cases, the epithelial cells are columnar and contain large nuclei. The stroma in these cases is somewhat larger than in the typical castrate state and several scattered atrophic uterine glands are evident (fig. 3). The lumen in all cases is small.

Vagina. Prominent and prolific stratification of the vaginal epithelium in the area of the vaginal orifice occurs in every case (figs. 5, 7, 9). The response is irregular, varying in intensity and absent in the portion of the vagina nearest the uterus (fig. 11). In the vicinity of the vaginal orifice numerous areas of atypical cornification are present. Long strands of keratinized materials are seen to be sloughing

off the outer layer of cells into the lumen (figs. 5, 6, 7). Deep proliferations of epithelial cells extend into the stroma of the vaginal wall (figs. 5, 7). The outermost cells in the epithelium of the stratified areas give evidence of undergoing necrosis, the cytoplasm is vacuolated and the nuclei pycnotic (fig. 6). Leucocytes are abundant in the stroma and are seen migrating through the epithelium into the vaginal lumen. Mitotic figures are frequently observed in the vaginal epithelium (fig. 8).

The vaginal muscularis shows a greater degree of development than in the ovariectomized control animals. Serial sections of the vagina indicate that level for level, stratification is present as compared with similar areas of the control animals. The epithelium of the ovariectomized controls is atrophic (fig. 10) in all areas of the vagina.

DISCUSSION

The results of the present work appear to indicate that the adrenal cortex elaborates an estrogenic factor, and that the nature of the response of the genital tract to this factor follows the pattern of a gradient. The most intensive reaction occurred in the area of the vaginal orifice and from there decreased progressively toward the uterus in which no observable response was noted. This finding compares favorably with the concept of varying thresholds of response of the genital tract to estrogenic stimulation. Brouha and Simonnet ('27) demonstrated that the genital tract of the rat does not follow the "law of all or none" response to estrogenic stimulation. Hemmingsen ('33) showed that the dose of estrin necessary for the induction of mating responses in spayed rats is larger than the dose sufficient to produce vaginal cornification. Marrian and Parkes ('30), Clauberg ('36) and Szarka and Kurtz ('38) demonstrated that a greater amount of estrogenic substance is required to produce uterine estrus than is required to produce vaginal cornification. In the castrate mouse (Meyer and Allen, '33) and castrate rat (Hartman, '44) subcornifying amounts of estrogen cause vaginal

mucification and increased amounts result in complete cornification of the vaginal mucosa. Evidence has been presented that uterine estrus in the rat is not always associated with vaginal estrus (Freed, Mesirow and Soskin, '37; Freed, Hechter and Soskin, '39).

Masculinization effects produced by androgens in female hamsters by Bruner and Witschi ('46) were most effective in those parts of the genital system most distant from the ovaries. The investigations of Littrell, Tom and Hartman ('46) on the time of opening of the vaginal membrane in weanling guinea pigs in relation to concentration of estrogen gives strength to the concept of a gradient or varying threshold type of response in that the average time of vaginal opening decreased with increased dosage.

The results of the present work are best explained by the threshold concept developed by these investigators. It would appear that the uterus has a higher threshold than the vagina and the opening of the vagina would appear to respond with the lowest threshold to estrogenic stimulation. At the beginning of sexual maturity, small amounts of estrogen seem to influence the vaginal orifice while the vagina is still of the immature type. A similar effect is noted in the sexual skin of monkeys which responds to slight doses of estrogen which do not influence other areas of the genital tract. The transplanted adrenal cortex in this experiment appears to be secreting estrogenic substances and it is plausible to assume that the area of the genital tract having the lowest threshold would respond. The pattern of response indicates a specific hormonal influence and precludes mechanical stimulation as a factor.

Another question is raised, however, as to whether the response is the result of a true estrogenic hormone, or whether the animals were affected by androgens and progesterone as well. The capability of the adrenal cortex to produce androgens (Burrill and Greene, '41a) and progesterone (Beall and Reichstein, '38) has been demonstrated. These two groups of steroids have been reported to have various effects on the

female reproductive accessories. Hisaw, Greep and Fevold ('37) showed that progesterone can modify and inhibit the action of estrogen in castrate monkeys. Courrier ('38) reports experiments which demonstrated the synergism or antagonism of progesterone and estrogen in the cat, rabbit and rat. Hartman ('44) noted that progesterone, while somewhat stimulating to the vaginal mucosa of the rat, has so little growth promoting property as compared to estrogen that it should not be referred to as estrogenic. Warren ('35) found pure crystalline androsterone to be non-estrogenic by the vaginal cornification test and Jones and Astwood ('42) found that androgens are effective in modifying the vaginal effect of estrogen. Korenchevsky and Dennison ('36) studied the effects of testosterone and estrone injected into ovariectomized rats, alone and simultaneously. Cornification of the vaginal epithelium was never attained with testosterone alone. In the present investigation, if androgens are elaborated by the transplanted adrenal cortex, the degree of cornification attained indicates that estrogens are also elaborated.

In the present work, the substances which act on the vagina are not identified, therefore, it is not possible to determine the relationship of activity between various adrenal steroids. There are no indications that the effects of progesterone, estrogen or androgen are additive or synergetic when not acting in optimal physiological dosages. On the contrary, when the vaginal tract is under simultaneous stimulation by these three groups of steroids, the evidence indicates an antagonistic or inhibiting reaction. Therefore, the partial stratification and incomplete cornification observed in this experiment may be due to either the low output of estrogen by the adrenal cortex or because in addition to estrogen the adrenal cortex under these experimental conditions may release progesterone and/or androgens the effect of which on the vaginal wall may be such as to condition it to become less responsive to estrogenic stimulation. In regard to hepatic inactivation as a factor, Burrill and Greene ('41b) demonstrated in the rat that adrenal androgen is not inactivated by the liver while Schiller ('45)

came to the same conclusion in the rat for adrenal estrogen. This evidence appears to indicate that hepatic inactivation is not a factor in preventing vaginal response to adrenal hormones in ovariectomized rats.

The absence of estrogenic stimulation of adrenal secretions in the ovariectomized animal may be due to the dilution of the hormone in the blood. The concentration of the hormone may become too small to elicit any response. Fee, Marrian and Parkes ('29) demonstrated that estrogen disappears from the blood very rapidly. The technique of transplantation employed in the present work permitted the cortical secretions to penetrate directly into the genital tract and therefore minimized the dilution factor. This technique was found to be effective by Katsh, Gordon and Charipper ('48) who demonstrated the andromimetic action of adrenal cortical transplants to the seminal vesicle of adult rats.

It is difficult to account for the persistent diestrus type of smear in the experimental animals in view of the features revealed by histological observations. Keratinized areas of the vaginal epithelium are revealed sloughing off, yet these were not observed in smears made by the lavage method. Admittedly, this atypical appearing cornification was not present in large amounts and it is quite possible that it was missed while observing the smears under the microscope.

The regenerated adrenal tissue which was implanted in a pocket in the wall of the uterus failed to show complete zonation. There was no difference in vaginal response noted between these animals and the group with adrenals transplanted to the wall of the uterus in which all three zones are apparent. This indicates that the estrogenic activity displayed by these transplants is possibly a property of relatively undifferentiated cortical cells. This is in agreement with the findings of Katsh, Gordon and Charipper ('48).

SUMMARY AND CONCLUSIONS

Adrenal cortical tissue was autografted to the uterus of ovariectomized rats in order to determine whether the se-

cretion products of these transplants could elicit estrogenic response in the genital tract. Histological studies showed stratification and apparent cornification of the vaginal epithelium in the area of the vaginal orifice. The reaction decreased progressively toward the uterus in which no observable response was noted. The type of response observed appears to follow the pattern of a gradient and is interpreted to be due to varying thresholds of response of the genital tract to estrogenic stimulation. The pattern of this varying threshold is described. The ability of the adrenal cortex to secrete androgens and progesterone in addition to estrogens, under the conditions of this investigation is discussed in relation to the concomitant influence of these hormones on the genital tract. The conditions of this experiment whereby the genital tract is brought directly under the influence of the adrenal cortical secretions eliminates the factor of dilution of the cortical hormone in the blood.

It is concluded that, under these conditions, the adrenal cortex secretes an estrogenic factor capable of eliciting a response in the area of the genital tract which has the lowest threshold.

LITERATURE CITED

- BEALL, D., AND T. REICHSTEIN 1938 Isolation of progesterone and allopregnanolone from the adrenal. *Nature*, 142: 479.
- BOURNE, G., AND S. ZUCKERMAN 1941 The influence of the adrenals on cyclical changes in the accessory reproductive organs of female rats. *J. Endocrinol.*, 2: 268-282.
- BROUHA, L., AND H. SIMONNET 1927 La folliculine et la "loi du tout ou rien" chez les femelles adultes. *C. R. Soc. Biol.*, 97: 686-688.
- BRUNEE, J., AND E. WITSCHI 1946 Hormonal modification of sex development in female hamsters. *Anat. Rec.*, 94: 367 (abs.).
- BURRILL, M. W., AND R. R. GREENE 1941a Androgen production in the female rat. *Endocrinol.*, 28: 871-873.
- 1941b The liver and the adrenal androgen of the rat. *Endocrinol.*, 28: 874-876.
- CLAUBERG, C. 1936 Stoeckels Handbuch für Gynakologie. Munich.
- COREY, E. L., AND S. W. BRITTON 1934 The ovarian cycle and the adrenal glands. *Am. J. Physiol.*, 107: 207-212.
- COURRIER, R. 1938 Les rapports fonctionnels des hormones ovariennes (In) *Les Hormones Sexuelles. Conférence du Collège de France. Comptes Rendus Publiés, par. L. Brouha. Paris, France.*

- FEE, A. R., C. F. MARRIAN AND A. S. PARKES 1929 The significance of the occurrence of oestrin in male urine. *J. Physiol.*, 67: 377-381.
- FREED, S. C., S. D. MESIROW AND S. SOSKIN 1937 On the composite nature of the estrus phenomenon. *Endocrinol.*, 21: 731-734.
- HECHTER, C., AND S. SOSKIN 1939 Inadequacy of the vaginal smear in the rat as an index of ovarian dysfunction caused by diet. *J. Endocrinol.*, 1: 268-274.
- HARTMAN, C. G. 1944 Some new observations on the vaginal smear of the rat. *Yale J. Biol. and Med.*, 17: 100-112.
- HEMMINGSSEN, A. M. 1933 Studies on oestrous-producing hormone (oestrin). *Skand. Arch. Physiol.*, 65: 97-250.
- HISAW, F. L., R. O. GREEP AND H. L. FEVOLD 1937 The effects of oestrin progestrin combinations on the endometrium, vagina and sexual skin of monkeys. *Am. J. Anat.*, 61: 483-504.
- JONES, G. E. S., AND E. B. ASTWOOD 1942 The physiological significance of the estrogen-progesterone ratio on vaginal cornification in the rat. *Endocrinol.*, 30: 295-300.
- KATSH, S., A. S. GORDON AND H. A. CHARIPPER 1948 The andromimetic action of adrenal cortical transplants to the seminal vesicle of the adult rat. *Anat. Rec.*, 101: 47-57.
- KORENCHEVSKY, V., AND M. DENNISON 1936 The histology of the sex organs of ovariectomized rats treated with male or female sex hormone alone or with both simultaneously. *J. Path. and Bact.*, 42: 91-104.
- LITTRELL, J. L., J. TOM AND C. G. HARTMAN 1946 Time of opening of the vaginal closure membrane in weanling guinea pigs in relation to concentration of estrogens. *Anat. Rec.*, 94: 367 (abs.).
- MARRIAN, G. F., AND A. S. PARKES 1930 The relative amounts of oestrin required to produce the various phenomena of oestrus. *J. Physiol.*, 69: 372-376.
- MEYER, R. K., AND W. M. ALLEN 1933 The production of mucified cells of the vaginal epithelium of certain rodents by oestrin and by corpus luteum extracts. *Anat. Rec.*, 56: 321-344.
- MOON, H. D. 1937 Effect of adrenocorticotropic hormone on the sexual development of spayed rats. *Proc. Soc. Exp. Biol. and Med.*, 37: 36-37.
- NELSON, W. O. 1941 Production of sex hormones in the adrenals. *Anat. Rec.*, 81: 97 (abs.).
- PARKES, A. S. 1945 The adrenal-gonad relationship. *Physiol. Rev.*, 25: 203-254.
- SCHILLER, J. 1945 Vaginal response to adrenal stimulation. *Proc. Soc. Exp. Biol. and Med.*, 60: 207-210.
- 1946 Differences in the action of crystalline and non-crystalline preparations of estriol. *Bull. Johns Hopkins Hosp.*, 79: 243-246.
- SZARKA, A. J., AND G. KURTZ 1938 A study of the ratio of the amount of theelin producing uterine and vaginal estrus. *Endocrinol.*, 23: 64-70.
- WARREN, F. L. 1935 Alleged oestrogenic activity of the male sex hormone. *Nature*, 135: 234.

PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures are photomicrographs of tissue fixed in Bouin's picro-formol solution and stained with Harris' hematoxylin and eosin.

1 Sixty-eight-day adrenal transplant to wall of uterus showing complete zonation of cortex. $\times 100$.

2 Fifty-seven-day regenerated adrenal tissue from portion of cortex implanted in wall of uterus. Lumen of uterine horn present. $\times 50$.

3 Same adrenal cortical tissue as in figure 2 showing both uterine horns. Adrenal tissue at lower left. $\times 50$.

4 Seventy-two-day adrenal transplant to wall of uterus. Uterus shows no sign of stimulation. $\times 50$.

5 Sixty-eight-day adrenal transplanted rat showing stratification and apparent cornification of vaginal wall near orifice. $\times 100$.

6 Same area as figure 5 under higher magnification. Epithelial cells appear vacuolated. $\times 440$.

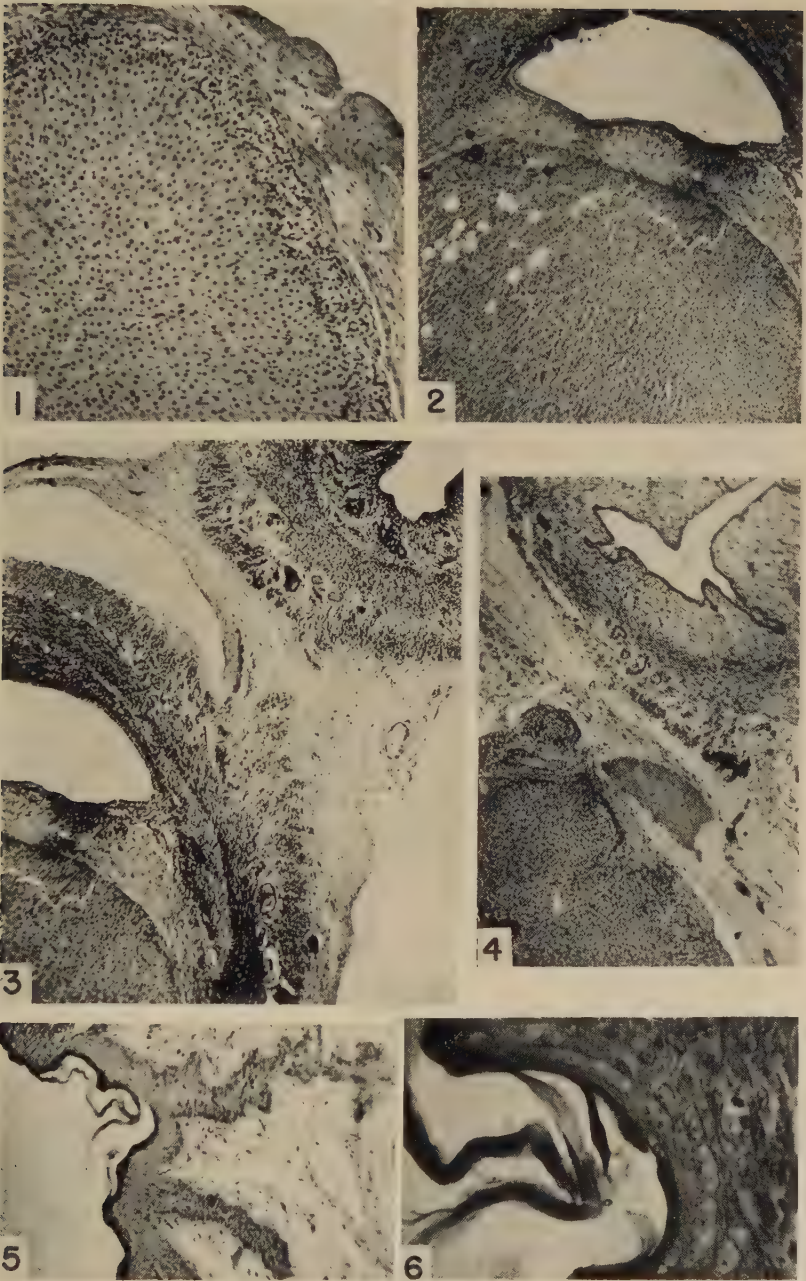


PLATE 2

EXPLANATION OF FIGURES

All figures are photomicrographs of tissue fixed in Bouin's picro-formol solution and stained with Harris' hematoxylin and eosin.

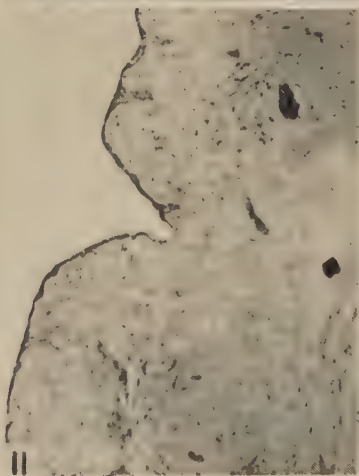
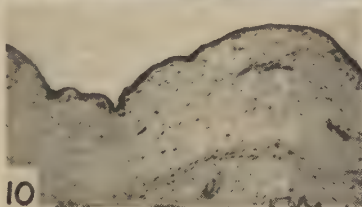
7 Seventy-two-day adrenal transplanted rat showing stratification and apparent cornification of vaginal wall near orifice. $\times 100$.

8 Seventy-two-day adrenal transplanted rat showing mitotic figure in vaginal epithelium. $\times 440$.

9 Seventy-two-day adrenal transplanted rat showing early stages of apparent cornification of stratified vaginal epithelium near orifice. $\times 100$.

10 Seventy-two-day ovariectomized control rat showing atrophy of vaginal epithelium near orifice. $\times 100$.

11 Seventy-two-day adrenal transplanted rat showing atrophy of vaginal wall near uterus. Epithelium resembles castrate type of ovariectomized control shown in figure 10. $\times 100$.



BRAIN CELLS FROM HUMAN FETUSES AND INFANTS, CULTURED IN VITRO AFTER DEATH OF THE INDIVIDUALS¹

MARY JANE HOGUE

*Department of Anatomy, School of Medicine,
University of Pennsylvania, Philadelphia*

FIFTEEN FIGURES

During the past 7 years human fetal brain cells have been studied by the tissue culture method in this laboratory. As the work progressed it became increasingly evident that the cells of fetal brains do not all die when the individuals die but continue to live in situ, sometimes for days, and can be cultured long after the heart has stopped beating and the individual has been pronounced dead.

MATERIAL AND METHODS

Only human material has been used. One hundred twenty-one brains of fetuses from 16 mm C R length to stillborn infants and brains from two infants that lived 1 day and 5 months respectively were studied.

The brains either were kept in the skull or were removed aseptically and refrigerated in Petri dishes, sometimes in 0.85% NaCl solution but often without fluid. Growth was obtained from explants regardless of how the brains were stored.

The temperature at which the brains were kept before culturing was important. If they were kept at 6°C. one was reasonably certain of getting some growth of cells up to the

¹Aided by a grant from the Faculty Research Fund of the University of Pennsylvania.

9th day after death. The few attempts to culture brain tissue from fetuses that had been dead for longer than 9 days (i.e., 12-14 days) were unsuccessful.

The parts of the brain used were cortex of frontal and occipital lobes, caudate nucleus, hippocampus, walls of the lateral ventricles, cerebellum and medulla oblongata. On a few occasions the dentate nucleus and vermis were removed from the cerebellum and cultured separately.

Explants about 1 mm in diameter were cut from these regions and cultured by the hanging drop and roller tube methods. The explants were fastened to the glass cover slip or to the sides of the test tubes with chicken plasma coagulated with chick embryo extract. A mixture of 6 ml of Tyrode solution and 6 ml of chick embryo extract to which were added 3 or 4 drops of human placental serum was used as a nutrient medium. A drop of this medium was added to each hanging drop culture every two to three days and about 12 drops were added to each roller tube culture every 3 or 4 days. In each case the old medium was removed before the new medium was added. The incubator was operated at 37.5°C.

OBSERVATIONS

The type of growth was similar in cultures made by both techniques. The first sign of life was the appearance of fine cell processes at the edge of the explant (fig. 1). Sometimes these were evenly distributed around the explant but they often appeared in isolated places quite far apart (fig. 9). Under certain conditions these processes were withdrawn. Changing the medium seemed to disturb the growth and the cell processes were drawn back to the explant. Later they might spread out again but usually they disintegrated and formed granules (Hogue, '46) which hindered the later migration of cells from the explant.

Transference of an explant to a new cover slip was occasionally made successfully, though always at the expense of the new growth surrounding it. When the explant had settled into the new coagulated drop of plasma it quickly resumed

its growth. In some experiments the explant was removed entirely; the cells that had moved out from it remained on the old cover slip and lived for long periods of time, showing that it was unnecessary to have the explant present to maintain the cultures.

After storage of tissue in the refrigerator, appearance of new growth was delayed. This delay was directly proportional to the time elapsing after death. The sooner the cultures were made after death the shorter was the lag period. For example, in cultures from a 165 mm fetus that had been dead two days, cells began migrating from some explants on the second day; while cultures from a 133 mm fetus that had been kept in the refrigerator for 7 days, showed no cells migrating from the explants until the 17th day of culturing.

Several varieties of cells migrated out from the explant. It was impossible to be certain of the identity of some of them but it is probable that neurons as well as many neuroglia were present. The question naturally arises as to how nerve cells can be distinguished from glia cells in cultures such as these. In one instance the culture was killed and stained and the large cells were observed to contain basophilic particles resembling Nissl bodies which are characteristic of neurons. In most of the experiments identification was attempted on the basis of other criteria.

After daily observations of some of the living cells for long periods certain characteristics strongly suggested that they were neurons. In the first place, many of them were much larger than any other cells, and could scarcely be glia or macrophages. There may have been many small neurons present, but these could not be distinguished from certain other cells in the living cultures. The cells believed to be neurons had processes that were larger and more branched than those of other cells. The cells thought to be neurons tended to separate from each other, while other types of cells, especially those identified as oligodendroglia, clung to one another and to the larger isolated cells.

A few types of large cells cultured from the cerebellum seemed quite probably neurons. Among these were some that had characteristics of Purkinje cells (Hogue, '47a). As they migrated from the explant they could be identified by their two large "stag-horn shaped" dendritic processes. However, when they became free from the mass of cell processes immediately around the explant these processes changed shape. From that time on it became impossible to identify these cells except by their great size. A few cells in cultures from the cerebellum were even larger than Purkinje cells. These had many branched processes coming from all parts of the body of the cell. They were not identified. Another type of cell frequently seen in cerebellar cultures had a small body and long processes which were branched near the ends (fig. 14). It had the shape of a brittle starfish. This cell could not be identified as any element characteristic of the intact cerebellum.

Since the large Purkinje cells were identified in cultures, even though they lost most of their characteristics as soon as they became free from the explants, it seems probable that a number of the other types of large, medium sized and even small cells freeing themselves from explants were in reality neurons. More than that, one cannot conclude at present.

Identification of neuroglia was somewhat easier than neurons. Oligodendroglia were usually the first cells to appear in brain tissue cultures. They could be identified as spherical bodies packed together in groups in the explants (Hogue, '47a). As they left the explants they developed fine processes at either end; there resulted a mesh work of cells and processes as shown in figure 3, or the little cells lined up along the long processes of other cells as shown in figure 4. Later when they had reached the outer part of the culture they remained in a mesh work, attached themselves to larger cells by their processes (fig. 10), or seemed to settle down in little depressions on the body or processes of a neuron (Penfield, '32; Hogue, '47a).

Russell and Bland ('33) have described these cells in cultures from gliomas; and Canti, Bland and Russell ('37) recorded their rhythmic pulsations in a time lapse motion picture. Recently Pomerat² has demonstrated them in cultures from adult human brains.

It was impossible to identify the different types of astrocytes in the living cultures from brains of human embryos. Other investigators (Canti et al., '37) have demonstrated them in gliomas. Macrophages were among the first cells to appear in the cultures. There was no difficulty in their identification. They were full of small fat globules (Hogue, '47a) and had broad transparent processes, free from droplets and appearing like a waving veil around the cell body.

The question may be raised whether any cells in the cultures from fetuses were glioblasts or neuroblasts. Some of the cultures made from younger fetuses showed a type of cells not found in cultures made from older fetuses. These cells migrated in a manner similar to fibroblasts, but were smaller and contained fairly large granules. They often formed plates and did not have long, thin processes. When these were numerous the explant was not surrounded by a halo of cell processes. Such cells would seem to be primitive; perhaps they had not yet differentiated into the elements that characterize the older fetus or newborn. That they may have been neuroblasts or glioblasts cannot be denied.

It is probable that most of the cultured explants of brain tissues yielded fibroblasts although these cells were not present in great numbers. When it came to trying to decide the identity of a medium sized brain cell with processes, the possibility that it may have been a fibroblast was not ignored.

Nests or rosettes of cells that have been described in cultures of other tissues, but not often seen in cultures of brain cells, were commonly encountered in cultures of ependyma (Hogue, '47b). Such rosettes were seen in some of the older cultures of the present series. They were believed to result

² A movie shown at a Department of Anatomy seminar, University of Pennsylvania.

from rapid multiplication of cells which remained in groups instead of migrating away from one another (possibly also to the accumulation of cells around some foreign body).

Most of the cells in brain tissue cultures contained no pigment. However, a few cultures were found in which cells contained dark pigment granules of unknown composition.

Movement of the cells presumed to be neurons occurred in the cultures. They were found to move more rapidly when they lay among the mass of cell processes surrounding the explant than when they were beyond it and relatively free. Outside this area of cell processes they flattened out and sent out numerous branched processes, varying in arrangement according to the type of cell.

Photomicrographs of one cell taken 4 or 5 days apart reveal some movement in reference to a foreign body on the cover glass or particles in the medium, as illustrated in figures 11, 12 and 13. It can be seen that the shape of the processes changed. The large brain cells appeared to be migrating much like amebae by sending out one or two processes and streaming slowly after them. When the cell was free from the surrounding cell processes many branched processes formed all around it and there was usually a long slender process, possibly the axon, at the end of the cell nearest the explant (Hogue, '47a).

Survival of brain cells after death is such a remarkable phenomenon that 6 illustrative examples are considered in the following paragraphs. They were chosen as representing different sized fetuses, the smallest one being designated Case 1. The others follow in sequence according to size or age. The length of time after death that they were cultured varied with the different fetuses.

Case 1. This fetus, 88 mm C R length (about $12\frac{1}{2}$ weeks) was received within an hour after it had been removed at therapeutic abortion. Hanging drop cultures were made from the cerebellum at once and the rest of the brain was placed in a refrigerator at 6°C. The following day the explants of cerebellum were surrounded by halos of fine cell

processes, similar to those seen in figure 1. On the fourth day these processes had branched and formed an interlacing mass containing oligodendroglia, other small cells and macrophages. On the 10th day cells had migrated beyond this mass and were moving about freely. By the 16th day there was a good growth of large granular cells which appeared to be neurons and on the 29th day the hanging drop was well covered with these elements and glia cells. Figure 3 shows the mass of interlacing processes containing oligodendroglia on the 22nd day. Figure 14 shows what appears to be a neuron with its several processes resembling a brittle starfish on the 35th day of culture. These cultures lived for 77 days.

Two days after the brain had been placed in the refrigerator, pieces were taken from the wall of a lateral ventricle and 10 hanging drop cultures were made from them. As good growth resulted as in the cultures made from the cerebellum immediately after delivery of the fetus. The cultures lived for 120 days.

After the brain had been in the refrigerator 5 days, 10 hanging drop cultures were made from the medulla oblongata. When these cultures were two days old there was little migration of cells, but after 7 days there were free cells, cords of cells and fine cell processes appearing in places around the periphery of the explant. On the 11th day the explants were completely surrounded by cells and processes of cells which had not yet emerged from the explant. These cultures lived for 46 days.

After the cerebral hemispheres had been in the refrigerator in a large Petri dish without fluid for 8 days a small piece was removed and washed in 0.85% NaCl solution. From this, 10 hanging drop cultures were made. During the first two days there was no sign of growth. On the 4th day fine cell processes, macrophages, cords of medium sized cells, oligodendria and even some large cells resembling neurons appeared. On the 8th day there was good growth of small and medium sized cells and fine cell processes forming a halo

around the explant; the elements resembling neurons had migrated far out in the medium. Figure 1 shows these cell processes and a few small cells emerging from the explant on the 14th day. Figure 4 made on the same day but from a different culture shows longer cell processes with small round or oval shaped oligodendroglia resting on them.

A week later there was excellent growth of large neuron-like cells, macrophages and glia in 5 of the cultures. In the other 5 cultures the explants had degenerated. Later 4 cultures became infected but one continued to thrive for 179 days at which time it was fixed in formalin and stained with thionin. The presence of small granules resembling Nissl material in the cytoplasm of some of the large cells supported the belief that these cells were neurons. Figure 5 shows one of these cells in the culture at 168 days.

Case 2. This 92 mm C R (about 13 weeks) fetus, from a therapeutic abortion, was received 15 minutes after the heart was said to have stopped beating. Roller tube and hanging drop cultures were made at once from the cerebellum. The roller tube cultures lived for 30 days and the hanging drop cultures for 75 days. Most of the cells which grew out from the explants were undifferentiated cells, though a few cells with processes were seen.

After the cerebral hemispheres had been stored in the refrigerator in Petri dishes without fluid for 9 days hanging drop and roller tube cultures were again made. The drop cultures did not grow but the roller tube cultures had good growths of undifferentiated cells, glia cells and one large cell that may have been a neuron. These cultures lived for 43 days when the experiment was discontinued.

Case 3. The next fetus measured 165 mm C R and was about 20 weeks old. It had been dead for two days before cultures were made. During the first 24 hours after death it was kept at 4°C. in the hospital refrigerator. It was then transferred to the laboratory and for the second 24 hours was kept at 6°C. Eighteen hanging drop cultures were made from the cerebellum. Table 1 shows how slowly growth began in

these cultures. Two of the cultures showed no signs of growth until the 14th day and 4 of the cultures did not grow at all. At the present time two of the original cultures are living at 220 days. Most types of cells can be found in them.

TABLE 1

Slow growth of cultures from a 165 mm C R human fetus (cerebellum)

10/6/49	Fetus died
10/7	Received and stored in refrigerator
10/8	Made 18 hanging drop cultures
10/10	2 cultures with cell processes 1 culture with free cells beyond the explant
10/12	4 cultures with free cells beyond the explant
10/14	7 cultures with free cells beyond the explant
10/17	12 cultures with free cells beyond the explant
10/20	14 cultures with free cells beyond the explant 4 cultures without growth

Case 4. A fetus measuring 195 mm C R, about 21 weeks old, had been dead two days when it was received. The brain tissue was in good condition as it had been kept at 4°C. in the hospital refrigerator. The cerebellum was cultured in hanging drops. On the 4th day glia cells and macrophages appeared. By the 13th day there were good growths of these elements. Purkinje cells were recognized on the 48th day. Some cultures became infected and were treated with potassium pencilling, without harm to the neurons which continued to migrate. The cultures lived for 188 days.

Case 5. A full term stillborn fetus weighing 2950 gm was received 24 hours after delivery, having been kept in the 4°-refrigerator during that time. Parts of the cerebellum were cultured in hanging drops. On the third day macrophages and cells resembling astrocytes appeared. On the 16th day large Purkinje cells were moving freely but very slowly in the cultures. A series of photomicrographs of one of these cells taken a few days apart is shown in figures 6, 7 and 8. Among the large cells, quite a few were binucleate and multinucleate. The cultures lived for 68 days.

Case 6. A newborn infant which had lived one day was received after it had been dead 5 days during which time it had been kept in the hospital refrigerator at 4°C. The dentate nucleus and parts of the cerebellar hemispheres were cultured in hanging drops and in roller tubes. These cultures exhibited slow growth, with the appearance first of fine cell processes, then macrophages and glia cells (fig. 2). On the 25th day large cells resembling neurons made their appearance. Purkinje cells were identified on the 45th day. Figure 10 was taken when the culture was 47 days old and shows a medium sized cell of unknown type and oligodendroglia grouped around it. Figure 15, taken on the 50th day, illustrates a medium sized stellate neuron-like cell. Some of these cultures are still in good condition after 323 days.

DISCUSSION

Until the present studies it has been generally believed that nerve cells die very soon after the individual dies. In 1922 Lewis and McCoy studied the survival of different kinds of cells after the death of white rats, guinea pigs, rabbits and man. They used as their criterion of life the presence of granules and vacuoles which took the neutral red dye. The white rat was the only animal whose brain cells were studied. Cells from the cerebral cortex and cerebellum rarely contained red granules or vacuoles. They found a few cells with pink granules and vacuoles 15 to 30 minutes after death. One hour later practically all cells were a diffuse red, indicating in their opinion, that the cells were dead. Lewis and McCoy suggested that death probably proceeded so fast that neutral red granules and vacuoles did not have a chance to form.

In 1937, Alvarez reviewed the literature on the subject of the survival of tissues after death and concluded that the injury done to nerve cells by anoxemia for 5-10 minutes is irreversible. He thought that the survival of nerve cells for a longer time showed that the experimenters had not stopped the circulation completely. In the tissue cultures of the present work there was no blood to circulate. However, small

amounts of human placental serum were added every 48 hours. Under these conditions cells identified as Purkinje cells and cells that were probably neurons of other types survived and exhibited activity.

Both Lewis and McCoy and Alvarez emphasize the importance of keeping tissues cool after death and agree with Buccianti ('31) who said that moderate cooling of tissues down to 0°C. lengthened the survival time of chick tissues.

Waterman ('39) worked with embryo rabbits which were put in covered dishes at low temperature. Parts of them were then transplanted to the omental bursa of an adult rabbit. These grafts were allowed to develop for 10 days and then removed for study. When he used pieces of cerebral hemisphere, cerebellum and spinal cord, he found that they showed evidence of disorganization and degenerative changes after 24 hours at 5°C. but he thought that low temperature was not as harmful as the separation from the blood supply.

Recently Murray and Stout ('47) while working with adult human sympathetic ganglion cells removed at operation, found that storing them in a refrigerator at 4°C. overnight did not kill the neurons though "the lag period before growth began was increased but the vitality of the cultures was hardly diminished." Their sympathetic nerve cells, so treated, lived in tissue cultures for 5 months.

Ferguson ('49) in a short review of literature on the subject of the effect of temperature on the survival of tissues outside the body says that most workers are agreed that there is an optimum temperature which varies with the tissue and the animal.

In the present paper the problem is limited to human brain tissues. In these experiments 4°–6°C. was found to be excellent for keeping brain tissues and nerve cells alive for at least 9 days.

Many investigators have cultured nervous tissue from chick embryos. When one compares the behavior of the neurons from human fetal brains with that of the neurons from the chick embryo spinal ganglia one difference is very evident: the neurons in the brain migrate away from the ex-

plant while those in the spinal ganglia usually remain in the ganglia after the nerve fibers, sheath cells and connective tissue cells have moved out. Such observations on ganglion cells have been reported by Weiss and Wang ('36), Meyer and Jablonski ('37), Murnaghan ('41) and Murray and Stout ('47).

Levi and Meyer ('41) think spinal ganglia neurons do not move because they did not see them change their shape. They suggest that the neurons at times were dragged away from the explant by the active migration of the fibrocytes. They also observed the flattening out of neurons in older cultures of spinal ganglia.

The sympathetic ganglion cells seem to behave more like the brain neurons. Murray and Stout ('47) reported the slow migration of human sympathetic ganglion cells and think the protoplasm of the sympathetic nerve cells is less solid than that of the spinal ganglia cells.

SUMMARY AND CONCLUSIONS

Living human brain cells including neurons and oligodendroglia were studied in tissue cultures made long after the individuals were pronounced dead. The time elapsing from death to the making of the tissue cultures varied from less than an hour to 9 days. The material came from fetuses from 16 mm C R length to full term stillborn infants and infants that lived one day and 5 months respectively. Some of the cultures have survived more than 323 days.

The longer the fetuses were refrigerated at 6°C., the longer was the lag period before the cells began to migrate from the explant into the culture medium. Movement of cells was followed and photographed from day to day. The cells resembling neurons moved faster while migrating through the mass of cell processes around the explant and more slowly when they reached the outer medium where they flattened out and formed many processes.

It was possible to identify a few neurons, and many oligodendroglia and macrophages with certainty. Purkinje cells

and glia were seen in the explants and observed to move out from them. In other instances neurons and glia were presumptively identified by shape, size and other characteristics.

The foregoing experiments demonstrated that nerve cells and glia cells from fetuses and stillborn infants do not all die when the individual dies. They can remain alive for a long period of time in refrigerated brain tissue and subsequently exhibit phenomena of growth in tissue cultures.

I am greatly indebted to Dr. Bachman and his staff at the University Hospital and to the Anatomical Board of the State of Pennsylvania for co-operation in obtaining material for this work.

LITERATURE CITED

- ALVAREZ, W. C. 1937 The survival of tissues after the death of an animal. *Quart. Rev. Biol.*, *12*: 152-164.
- BUCCIANTI, L. 1931 Sulla sopravvivenza alle basse temperature (fino a -25°) dei vari tessuti di embrioni di pollo di cui fu interrotta l'incubazione. *Archiv. f. Zellforsch.*, *11*: 397-423.
- CANTI, R. G., J. O. W. BLAND, AND D. S. RUSSELL 1937 Tissue culture of gliomata. Cinematograph demonstration. *Proc. Assoc. Res. Nerv. Ment. Dis.*, *16*: 1-24.
- FERGUSON, J. 1949 Low temperature and survival of embryonic tissue. *Sci.*, *110*: 47-48.
- HOGUE, M. J. 1946 Tissue cultures of the brain. Intercellular granules. *J. Comp. Neur.*, *85*: 519-530.
- 1947a Human fetal brain cells in tissue cultures: their identification and motility. *J. Exp. Zool.*, *106*: 85-108.
- 1947b Human fetal ependymal cells in tissue cultures. *Anat. Rec.*, *99*: 523-529.
- LEVI, G., AND H. MEYER 1941 Nouvelles recherches sur le tissu nerveux cultivé in vitro. Morphologie, croissance et relations récéproques des neurones. *Arch. de Biol.*, *52*: 133-278.
- LEWIS, W. H., AND C. C. MCCOY 1922 The survival of cells after the death of the organism. *John Hopkins Hosp. Bulletin*, *33*: 284-293.
- MEYER, H., AND W. JABLONSKI 1937 Cultivation of nerve cells in vitro over a long period. *J. Anat.*, *72*: 62-65.
- MURNAGHAN, D. 1941 Studies on living spinal ganglion cells. *Anat. Rec.*, *81*: 183-203.
- MURRAY, M. R., AND A. P. STOUT 1947 Adult human sympathetic ganglion cells cultivated in vitro. *Am. J. Anat.*, *80*: 225-273.
- PENFIELD, W. 1932 Cytology and cellular pathology of the nervous system. Hoeber, New York. Vol. 2, Sec. 9., p. 439.

- RUSSELL, D. S., AND J. O. W. BLAND 1933 A study of gliomas by the method of tissue culture. *J. Path. and Bact.*, 36: 273-283.
- WATERMAN, A. J. 1939 Survival of rabbit embryonic tissue after removal from the uterus and exposure to low temperature. *Anat. Rec.*, 73: 243-255.
- WEISS, P., AND H. WANG 1936 Neurofibrils in living ganglion cells of the chick cultivated in vitro. *Anat. Rec.*, 67: 105-117.

PLATE 1

EXPLANATION OF FIGURES

All the photomicrographs of living tissue cultures of human brain cells were made as routine records with a Leitz camera using 35 mm film.

1 Fourteen-day-old culture showing fine cell processes emerging from an explant of the cerebral hemisphere from an 88 mm fetus. Cultured 8 days after death. $\times 188$.

2 Fifty-day-old culture showing fine cell processes, small cells and one macrophage emerging from an explant of the cerebellum from an infant which had lived one day. Cultured 5 days after death. $\times 188$.

3 Twenty-two-day-old culture showing a mass of cell processes, oligodendroglia and a large cell (in the center) from the cerebellum of an 88 mm fetus. Cultured at once after a therapeutic abortion. $\times 188$.

4 Twenty-day-old culture showing cell processes and oligodendroglia from the same 88 mm fetus. Cultured two days after death. $\times 188$.

5 One-hundred-sixty-eight-day-old culture showing a cell resembling a neuron and other cells from the cerebral hemisphere of the same 88 mm fetus. Cultured 8 days after death. $\times 188$.

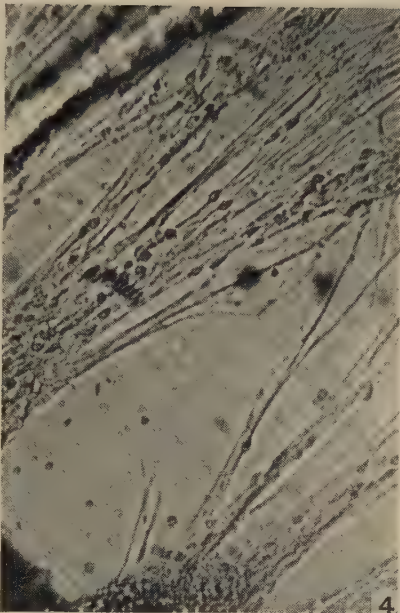
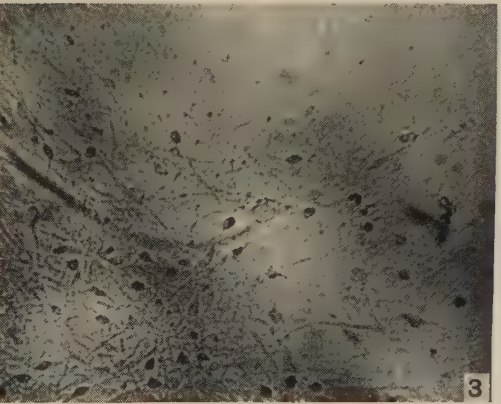
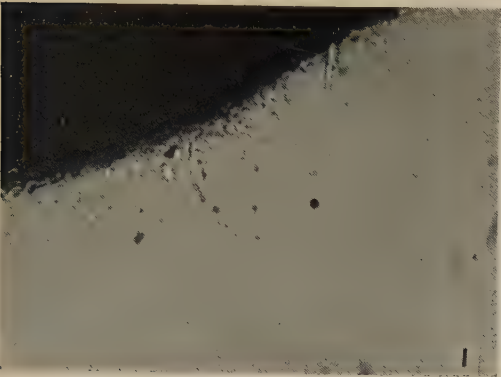


PLATE 2

EXPLANATION OF FIGURES

6 Twenty-six-day-old culture showing a Purkinje cell from cerebellum of full term newborn infant. Cultured one day after death. $\times 188$.

7 Same cell when the culture was 33 days old. $\times 188$.

8 Same cell when the culture was 35 days old. $\times 188$.

9 Thirty-four-day-old culture showing cell processes in spray formations. The explant was taken from the wall of the lateral ventricle of the cerebrum of an 88 mm fetus. Cultured two days after death. $\times 75$.

10 Forty-seven-day-old culture showing a medium sized cell of unknown type and many oligodendroglia from cerebellum of infant that lived one day. Cultured 5 days after death. $\times 188$.

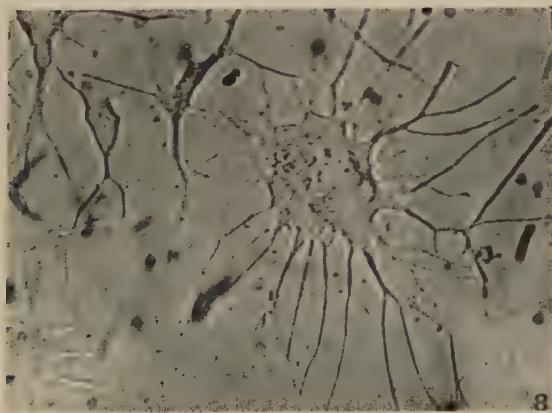
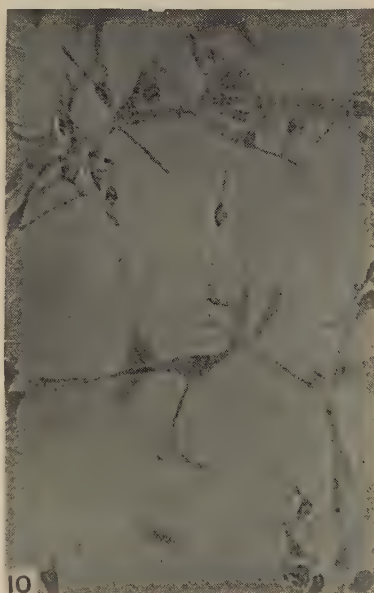
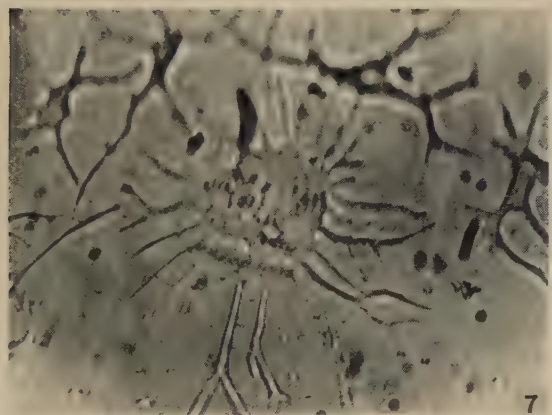
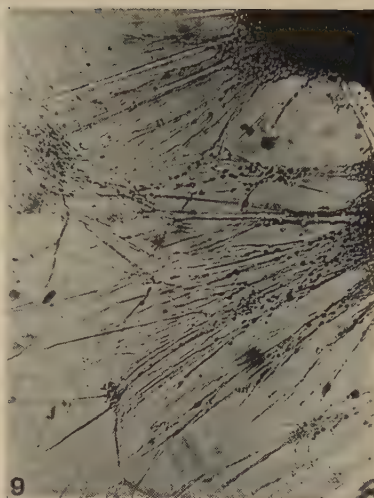
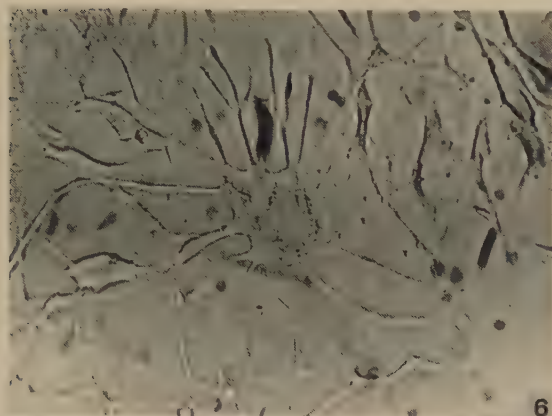
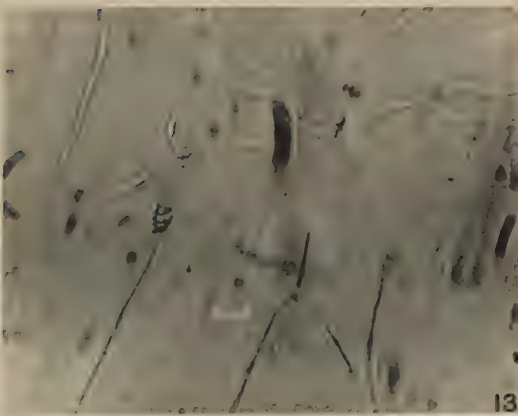
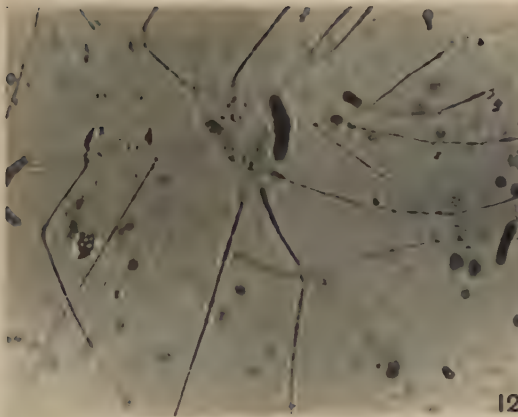
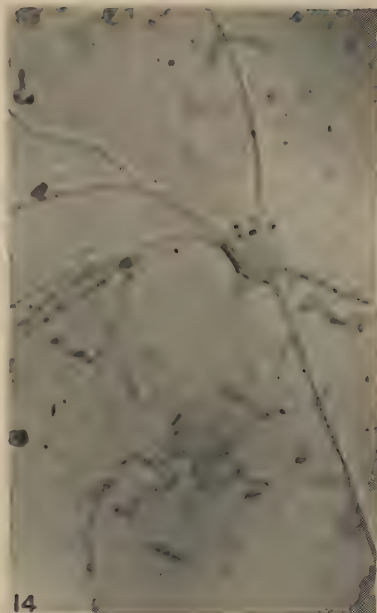
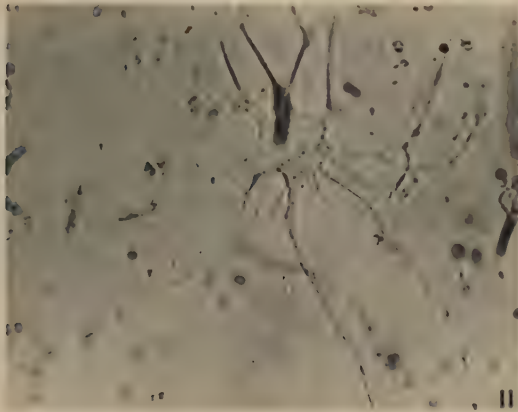


PLATE 3

EXPLANATION OF FIGURES

- 11 Nineteen-day-old culture showing a Purkinje cell from the cerebellum of an infant that lived one day. Cultured 5 days after death. $\times 188$.
- 12 Same cell when the culture was 26 days old. $\times 188$.
- 13 Same cell when the culture was 28 days old. Note movement of cell in reference to dark sausage-shaped spots of fixed position in figures 11, 12 and 13.
- 14 Thirty-five-day-old culture showing a cell resembling a neuron from cerebellum of 88 mm fetus. Cultured right after therapeutic abortion. $\times 188$.
- 15 Fifty-day-old culture showing a cell resembling a neuron from cerebellum of an infant that lived one day. Cultured 5 days after death. $\times 188$.



ANOMALOUS OPTIC CHIASMA IN FUNDULUS EMBRYOS

JANE M. OPPENHEIMER

*Department of Biology, Bryn Mawr College, and Osborn Zoological
Laboratory, Yale University*

FIVE FIGURES

INTRODUCTION

Total decussation of the fibers of the optic nerve is one of the most constant characteristics of the nervous system of teleost fishes. It is accordingly of considerable interest that two striking anomalies of the chiasma have occurred recently among experimentally treated *Fundulus heteroclitus* embryos. The two abnormalities appeared in material widely different in nature. They deserve description for any possible ideas they may suggest on the nature of the factors controlling the direction of outgrowth of the nerve fiber, which are in many ways no more clearly understood now than when Harrison ('14) first demonstrated stereotropism as a primary determining factor.

MATERIALS

The two embryos to be described derived from different series of experiments. One of them, which was cyclopic, was an embryo in which a graft of 180° germ-ring had been implanted into the anterior embryonic shield during the early gastrula stage, according to methods already described (Oppenheimer, '38). The other anomalous embryo, in which the eyes were normal in position, appeared in a series of embryos treated with metrazol (Oppenheimer, '50). The particular embryo in question had been dechorionated and

immersed in 2% metrozol at stage 23 (Oppenheimer, '37), allowed to remain in it for $17\frac{1}{2}$ hours, and was then removed at stage 24 to sea-water in which it continued development for 9 days through to stage 31, the final prehatching stage. Both of the embryos described in this communication were fixed in Bouin's solution, cleared in oil of wintergreen, sectioned at 10μ and the sections impregnated with silver by Power's ('43) modification of the Bodian technique.

DESCRIPTION OF EMBRYOS

Cyclopic embryo. In this embryo the two eyes, fused posteriorly, are located directly ventral to the brain; each of them gives origin to a large optic nerve which joins the diencephalon ventrally. The nerves, however, instead of decussating totally as is typical of the teleostan chiasma, both send some of their fibers to the ipsilateral and some to the contralateral side of the brain. The nerve from the left eye seems to send approximately half of its fibers to each side of the brain (fig. 1 and 2, representing two adjacent sections; the left nerve is seen on the right in the photomicrographs). The nerve from the right eye is seen in figure 3 in the section following immediately posterior; examination of this section at higher magnification (fig. 4) demonstrates that this nerve sends more fibers contralaterally than ipsilaterally. The three sections illustrated contain the whole chiasma. Once the fibers have entered the brain proper, they follow the usual course, ascending posterolaterally along the diencephalon to terminate in the optic tectum.

Metrazol-treated embryo. In this embryo both eyes are normal in size and position, and the anomaly involves primarily the optic chiasma. The optic nerve of the right eye in this case has failed to cross contralaterally and all its fibers pass to the ipsilateral side of the brain (fig. 5; the right nerve is seen on the left of the figure). In the section following immediately posterior to the one photographed, all the fibers from the left eye are seen to have crossed to the opposite side of the brain in typical fashion; they also pass, therefore, like

those of the right nerve to the right side of the brain. The fibers of the two nerves take their course together along the right side of the diencephalon dorsalwards to their termination in the right optic tectum.

Paper models of the left and right optic tecta of this embryo were constructed¹ according to the method worked out for *Fundulus* by White ('48). The weight of the model of the left tectum was 0.6950 gm as opposed to 1.0014 gm for the right, a difference of 0.3064 gm between the models of the two lobes. Thus the volume of the left optic tectum was 31% less than that of the right, a figure which may be compared to the average reduction of 19% (P.E. 1.05) reported by White as a result of removal of one eye at stages prior to the formation of the optic nerve. As in White's material, the reduction in the white matter was considerably more than in the gray. The model of the gray on the left weighed 0.4327 gm, as contrasted to 0.5370 gm for the right, a difference of 0.1043 gm representing a reduction of 20% for the gray. The model of the white matter on the left weighed 0.2606 gm, as opposed to 0.4620 gm for the right, a difference of 0.2014 gm and a reduction therefore of 43% for the white.

DISCUSSION

These embryos may shed some light on the nature of the factors directing the outgrowth of the optic nerve. In the case of the cyclopic embryo, it is possible that the ventral rather than the usual lateral location of the eyes was perhaps responsible for the anomalous crossing of the fibers. The more ventral location of the eye, by bringing about unusual spatial relationships of the developing nerves to each other and to the brain, may have altered secondarily the time, absolute or relative, of arrival of the fibers at particular destinations. If such mechanical factors as the selective adhesiveness and consequent selective fasciculation postulated by Weiss ('41) play their role in guiding fibers, such changed

¹I am grateful to Miss Isabel Kellers for the preparation and weighing of the paper reconstruction.

temporal relations might well effect changed connections within the central nervous system such as here described. If this is the case, experimental translocation of the eye in a ventral direction, accompanied by measurement of the outgrowth of the nerves at particular times, might provide a new mode of attack on the problem.

Clues to the explanation of the diversion of the right nerve to the right side of the brain in the case of the metrazol-treated embryo are even more elusive. As already reported (Oppenheimer, '50) this embryo was treated with the drug at a period during which the optic nerve was developing. If it could be postulated that in *Fundulus*, as in the amphibian tadpoles treated with the drug by Speidel ('40), the effect is exerted on the developing nerve endings which first degenerate and subsequently regenerate after removal from metrazol, one might expect abnormal connections or diversions in the routes of the nerves, especially if fiber tracts affect each other mutually during development. It is a curious fact that with the exception of two or three embryos, treated at about the same stage as the embryo described here, in which no optic nerve formed, this single embryo out of 274 studied histologically was the only one in the metrazol-treated series in which any abnormality of connections was detected.

One of the most striking aspects of this material is the constancy of the course of the nerve within the brain. No matter which the side of entry, all the constituent fibers of the anomalous nerves follow the typical path to the tectum, and the quantitative response of the tectum surely suggests the connection to be a functional one. It seems clear that whatever the factors are that serve as guides, they act with more constancy and stability within the central nervous system than between retina and diencephalon. It is conceivable that this might be related to the fact that there is greater homogeneity of material within the central nervous system than outside of it. If so, it is possible that further clues might be obtained by exploring experimentally some of the differences which distinguish the central nervous system from the areas im-

mediately surrounding it, or perhaps even those differences which characterize the lateral as opposed to the ventromedial diencephalon at the time the fibers are coursing past them.

SUMMARY

Two experimentally treated *Fundulus heteroclitus* embryos are described which exhibit unusual anomalies of the optic chiasma. In one, an operated embryo which became cyclopic, both optic nerves have divided to send part of their fibers to each side of the brain. In the other, an embryo treated with metrazol at the time of outgrowth of the optic nerve, both left and right optic nerves have entered the right side of the brain to terminate in the right optic tectum. In this embryo the volume of the left optic tectum is 13% less than that of the right.

Some factors are discussed which may possibly have some bearing on the abnormal course of the nerves. The fact that once within the brain the aberrant fibers follow the normal course suggests a great constancy and stability of the directive agents within the diencephalon than between retina and the point of entrance of the nerves into the brain.

LIST OF REFERENCES

- HARRISON, R. G. 1914 The reaction of embryonic cells to solid structures. J. Exp. Zool., 17: 521-544.
- OPPENHEIMER, J. M. 1937 The normal stages of *Fundulus heteroclitus*. Anat. Rec., 68: 1-15.
- 1938 Potencies for differentiation in the teleostean germ-ring. J. Exp. Zool., 79: 185-212.
- 1942 The decussation of Mauthner's fibers in *Fundulus* embryos. J. Comp. Neur., 77: 577-587.
- 1950 The development of *Fundulus heteroclitus* embryos in solutions of metrazol. J. Exp. Zool., 113: 65-85.
- POWER, M. E. 1943 The brain of *Drosophila melanogaster*. J. Morph., 72: 517-559.
- SPEIDEL, C. C. 1940 Studies on living nerves. VI. Effects of metrazol on tissues of frog tadpoles with special reference to the injury and recovery of individual nerve fibers. Proc. Am. Philos. Soc., 83: 349-378.

- WEISS, P. A. 1941 Nerve patterns: the mechanics of nerve growth. *Growth* (suppl.), 5: 163-203.
- WHITE, E. L. 1948 An experimental study of the relationship between the size of the eye and the size of the optic tectum in the brain of the developing teleost, *Fundulus heteroclitus*. *J. Exp. Zool.*, 108: 439-469.

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs prepared by Mr. E. Richard Deats of Philadelphia.

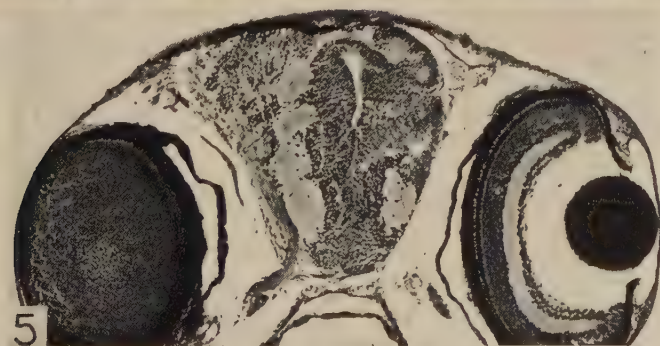
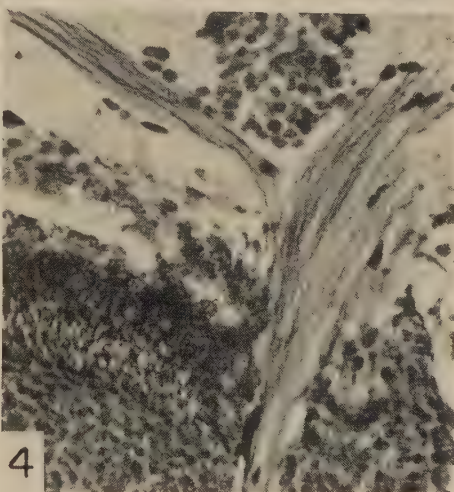
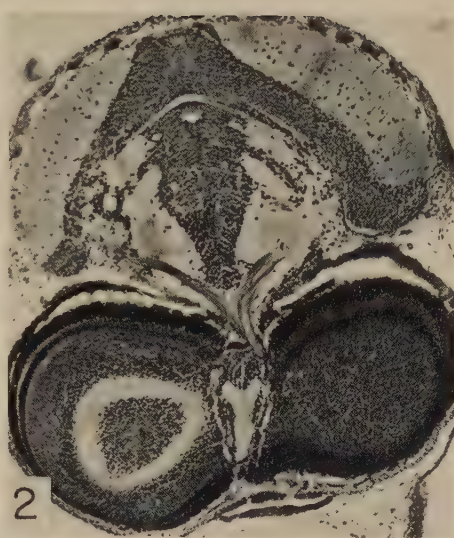
1 through 4 Sections through the optic chiasma of cyclopic *Fundulus* embryo. Bodian preparation.

1 and 2 Consecutive sections through the left optic nerve (right in the figure) which divides its fibers between the left and right sides of the brain. $\times 100$.

3 Section immediately posterior to that shown in figure 2, passing through the right optic nerve (left in the figure). $\times 100$.

4 Region of the chiasma from figure 3 at higher magnification, showing the unequal division of fibers between the left and right sides of the brain. $\times 440$.

5 Section through the metrazol-treated embryo described in the text, showing the right optic nerve (left in the figure) entering the right side of the brain. In the section immediately posterior, the left nerve enters the right side of the brain also. Bodian preparation. $\times 100$. Reproduced by courtesy of the *Journal of Experimental Zoology*.



BOOK REVIEW

RACES . . . A STUDY OF THE PROBLEMS OF RACE FORMATION IN MAN. By CARLETON S. COON, STANLEY M. GARN, AND JOSEPH B. BIRDSSELL 1950 xiv + 153 pages, 11 figures, 15 plates. \$3.00. Charles C. Thomas, Springfield, Illinois.

This monograph is the first dealing with physical anthropology in the American Lecture Series. The editors (T. D. Stewart, A. H. Schultz and W. W. Howells) had planned to split the field into clear-cut subdivisions and to have specialists present the facts in each, theory and speculation being subordinate. Doctors Coon, Garn, and Birdsell, finding this program too restrictive, have written on the topics in which they are currently most interested, namely, race formation in man, and the result is a most stimulating, though largely speculative, volume. Yet even the lay reader will recognize when facts end and speculation begins.

At the outset, race is defined, and the reader is informed that human races, like animal or plant species, are generally adapted to their environments. As usual, the paucity of exact observations in physical anthropology is deplored, the possibility of a relationship between soma and psychology is noted, and a brief word is said about the interpretation of statistical data. The second chapter, *Race and the Genes*, estimates the total number of genes in man, defines genotype and phenotype, points out that all races have the majority of their genes in common, and notes that similar visible characters may be due to different genes. A brief statement regarding blood group genes follows; mutation and selection are discussed. As factors influencing selection, the authors then say a word about human breeding habits, social selection, isolation and mixture and the barriers of caste.

After filling in the necessary background, the real meat of their subject is given. They discuss the ways in which man adapts himself to his environment, and the direct effects of the latter on the human organism. It is seen that some racial differences may thus be determined, whereas others are genetically produced, the distinction not always being easy to make. They further make it clear that environment does not produce those genetic changes which facilitate adaptation, but that it favors certain genetically determined char-

acters and tends to eliminate others. Particularly interesting is the discussion of adaptation, via selection, to desert conditions or to extreme cold. Desert dwellers tend to have a large surface relative to volume, whereas the reverse obtains in the Arctic. The former often have salient features; the faces of the latter are flat and an epicanthic screen may help protect the eyes. Nothing, however, is said about sweat glands. The significance of pigmentation, and the conditions which may increase or decrease it, are discussed, and there is an interesting section on hair form. By way of illustration, a few words are devoted to the races, which have become differentiated in consequence of these selective factors.

Chapter 7 considers the survival of archaic forms among living races, gives a brief comparison of man and other primates, and lists the criteria of evolutionary status in hominid evolution. A short paragraph on the cephalic index is appended. The reviewer is glad to see this index de-emphasized, but feels that the authors, in throwing out the contaminated bath, may have rejected a viable infant, which may still have a modest anthropological function. The next chapter discusses the selective effects of disease.

The final chapter presents a racial classification. It is noted that this is based on the phenotype. As a preliminary, several major groups are chosen, which the authors regard as *stocks*, each consisting of a number of races which seem similar to each other. These are the Negroid, Mongoloid, White, Australoid, American Indian, and Polynesian. These, say the authors, are not races. The definition of race may be reduced to this: "A race is a somatically unique population or collection of identical populations." Types are also distinguished from races and from stocks.

There follows a tentative classification of races. There are 30, beginning with the Murrayian, of Australia, and ending with the Neo-Hawaiian. Included are the traditional Nordic, Mediterranean and Hamite, and also the North American Colored.

All these topics are taken up in 112 rather meager pages of actual text, excluding the 26 excellent illustrations, most of which are full page. One feels sure that the authors would have welcomed less restriction. But this is a "lecture" series. Most of the topics could not be fully discussed and, no doubt, there was much omission. The importance of the book is that certain important selective factors are highlighted. It should serve to stimulate more intensive study, and it should help in orientation. It is regrettable that the most suggestive evidence for a very striking selective action of disease is omitted, namely, the contrast between the peripheral belt and the highlands of Madagascar, discussed by Ralph Linton. The least satisfactory

section is that dealing with the genes. On page 15 it is said: that "Selection alone cannot produce changes of race or species; new genes must appear from which selections can be made." This is doubtful with respect to races, since isolated segments of a polymorphic species may become distinct through the operation of selection and genetic drift. The reviewer feels that the discussion of mutation rates on page 16 is not clear. The reader may not grasp the fact that, in a given species, some mutations recur more frequently, others less frequently, whereas others are so rare that they may be regarded as non-recurrent. The volume published at Princeton in 1949 on Genetics, Paleontology and Evolution by Jepsen, Mayr, Simpson and others, can be recommended in this connection.

In conclusion, the reviewer would like to express his profound dissatisfaction with racial classification in general. It is a particularly difficult field in man because of the universality and complexity of hybridization. There is no satisfactory definition of race in man. Coon, Garn, and Birdsell, for example, list the Neo-Hawaiian, a "blend in process of formation and stabilization . . . with various Asiatic Mongoloid, European and American White, and diffuse Negroid elements added to the Polynesian." As races are defined, this may become a race, given isolation and panmixia. No doubt indubitable races have arisen from complex mixtures, the Polynesian, for example. And in Australia, another mixture, if less complex, is listed with the stocks. As in zoological taxonomy, the splitters and the lumpers are busy. Lumping is unsatisfactory, since it leaves too many groups undefined. On the other hand, the logical end of splitting, the reviewer is convinced, is the establishment of 2 billion races, more or less. Hardly any two individuals, barring identical twins, share all the same genes. No boundaries, upon which splitters and lumpers will agree, can be established. Should, therefore, physical characters be abandoned and serological factors alone be used as a basis of classification? It may be safely predicted that such a classification will be no more satisfactory than present ones. Numerous intermediate groups will appear. It may satisfy some if the population is defined simply in terms of the percentages of all the serological and other characteristics which it possesses and, perhaps, given a name. But, unless the reviewer is mistaken, what we are interested in is human evolution. Who and what were the antecedents of these people whom we call a race? To what extent, if any, are they related to some other race? What, precisely, is the nature of that relationship? If the two possess a certain proportion of common ancestry, due to hybridization, to what extent has selection tended to cause differential loss of genes derived from the common ancestor? If this

question can receive an answer, then can anything be inferred regarding the selective values of certain genes in certain environments? Hooton, in "Up from the Ape," has the right approach. At some time, presumably, there were primary races. Perhaps it is not possible today to learn what these were—an ancient secondary race may appear to be primary. At any rate, if it becomes possible to say that a given population is descended from certain apparently primary races, hybridized at specified times in specified percentages, and that selection, drift, and mutation have produced certain definable changes, then the concept of race may have a larger meaning. The problems which Drs. Coon, Garn, and Birdsell consider are central, if answers to the foregoing questions are to be obtained.

RICHARD ASHMAN

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PROGRAM FOR THE
FORTY-SEVENTH ANNUAL MEETING OF THE
AMERICAN SOCIETY OF ZOOLOGISTS

DECEMBER 27, 28, 29, 30, 1950

The Society will meet in conjunction with Section F of The American Association for the Advancement of Science, and in association with other biological societies. Most meetings for the presentation of papers and all symposia, except the one on "Radiobiology," will be held in Hotel Hollenden in Cleveland. One session for the reading of papers and the General Demonstration program will be held in the Biological Laboratories of Western Reserve University at 2080 Adelbert Road. The Demonstrations by Motion Pictures will be held in the auditorium of The Cleveland Academy of Medicine at 2009 Adelbert Road which is about one-half block from the Biology Building at Western Reserve University. The symposium on "Radiobiology" and the commercial exhibits will be held in The Cleveland Public Auditorium which is about two blocks from Hotel Hollenden on East 6th Street.

As one of the special features of the meeting Dr. Romer has organized a symposium on "Transition from Aquatic to Land Life." Our Society will co-sponsor the Friday portion of the symposium on "Radiobiology" at which time papers dealing with non-clinical aspects of the problem will be presented. Dr. Calvin S. Hall has organized a symposium on "Genetics and Behavior" as the A.A.A.S. Zoological symposium which will also be co-sponsored by our Society.

The annual dinner of the American Society of Zoologists to which all Zoologists and friends are invited, will be held at 6:00 P.M. on Friday, December 29th, in the Assembly Room of the Hotel Hollenden. Dr. L. V. Domm, Vice-President of the

American Association for the Advancement of Science and Chairman of Section F, will deliver the annual address on "Sex differentiation: Genes or Hormones."

The Biologists' Smoker, which will be sponsored by the A.A.A.S. and our society, will be held on Friday evening in South Exhibition Hall, Cleveland Public Auditorium, at 9:30 P.M.

One of the highlights of the meeting will be the excellent exhibits by commercial companies in the Auditorium.

Members are urged to register with the A.A.A.S. at the registration desk in Parlor H in the Hotel Hollenden as soon as they arrive in Cleveland if they have not already done so by mail.

As in previous years, the Society is highly indebted to the Wistar Institute of Anatomy and Biology for the prompt publication and mailing of this program.

LOCATION OF MEETINGS

Hotel Hollenden, which will be the headquarters of the Society, is located at Superior Avenue and East 6th Street and is within easy walking distance from the Terminal Depot at Public Square. There is frequent bus and street car service out Euclid Avenue from the Public Square, with a stop at East 6th Street. The Hollenden Hotel is a short half block north on East 6th Street.

It is recommended that members entering Cleveland on the Pennsylvania Railroad get off at the downtown Station at West 9th Street and Lakefront, unless they are staying at an uptown hotel, in which case it would be more convenient to get off at the Pennsylvania Station at Euclid Avenue and East 55th Street.

The Cleveland Public Auditorium, where the symposium on Radiobiology and the commercial exhibits will be held, is located two blocks from Hotel Hollenden at Lakeside and East 6th Streets.

The Western Reserve University Campus, where the Demonstration and Movie Programs will be held, is nearly 5 miles east from the Public Square, on Euclid Avenue. Transportation is adequate. There are several express bus lines and a street car line which give service every few minutes from the Public Square out Euclid Avenue. These stop at Adelbert Road, in the heart of the University Campus. They may be boarded on Euclid Avenue at East 6th Street. *The busses are marked:* 28 Windermere, 28 Avalon, 28 East 222nd Street, 30 East 140th — Coit, 30 Haydu, 45 Mayfield. *The street cars are marked:* Euclid — Windermere.

DINNER TICKETS

Tickets for the Zoologists' dinner on Friday evening, will be on sale at the A.A.A.S. registration desk in Parlor H in Hotel Hollenden at cost of \$3.10. The dinner will be held in the Assembly Room of Hotel Hollenden at 6:00 P.M.

It will greatly help those responsible for dinner arrangements if all who plan to attend will purchase their tickets as soon after they arrive in Cleveland as possible.

P R O G R A M

WEDNESDAY, DECEMBER 27TH

- | | |
|-----------|---|
| 2:00 P.M. | Symposium on " <i>Transition from Aquatic to Land Life</i> ,"
Dr. A. S. Romer, presiding. Ballroom, Hotel Hollenden. |
| 4:30 P.M. | Annual Business Meeting of Section F, A.A.A.S., Ballroom,
Hotel Hollenden. |
| 4:40 P.M. | Annual Business Meeting of the American Society of Zoologists. |
| 8:00 P.M. | Symposium on " <i>The Role of Taxonomy in Modern Zoology</i> " arranged by the Society of Systematic Zoology.
Ballroom, Hotel Hollenden. |

THURSDAY, DECEMBER 28TH

- | | |
|-----------|--|
| 9:00 A.M. | Section A. <i>General Physiology</i> . Papers 4-16. Ballroom, West half,
Hotel Hollenden. |
| | Section B. <i>Embryology</i> . Papers 17-27. Ballroom, East half, Hotel
Hollenden. |

THURSDAY, DECEMBER 28TH (continued)

- Section C. *Endocrinology*. Papers 28-38. Assembly Room, Hotel Hollenden.
- Section D. *Cytology and Protozoology*. Papers 39-47. Cypress Room, Hotel Hollenden.
- 2:15 P.M. Section A. *Cellular Physiology*. Papers 48-57. Room 44, Biology Building, Western Reserve University.
- Section B. *General Demonstrations*. Papers 58-73. Room 32, Biology Building, Western Reserve University.
- Section C. *Demonstrations by Motion Pictures*. Papers 74-80. Auditorium of the Cleveland Academy of Medicine, 2009 Adelbert Rd. (near Biology Building, Western Reserve University).

FRIDAY, DECEMBER 29TH

- 9:00 A.M. Section A. *Animal Biology and Sociobiology*. Papers 81-93. Ballroom, Hotel Hollenden.
- Section B. *Endocrinology*. Papers 94-99. Assembly Room, Hotel Hollenden.
- 9:30 A.M. Section C. Symposium on "Radiobiology." Ballroom, Cleveland Public Auditorium.

Special Event

For Animal Behaviorists and Sociobiologists

- 12:30 P.M. Luncheon and Business Meeting; Committee for the Study of Animal Societies Under Natural Conditions; Hotel Hollenden.
- 2:00 P.M. Symposium on "Genetics and Behavior." Ballroom, Hotel Hollenden.
- Symposium on "Radiobiology." Ballroom, Cleveland Public Auditorium.
- 6:00 P.M. Zoologists' Dinner, Assembly Room, Hotel Hollenden.
- 9:30 P.M. Biologists' Smoker, South Exhibition Hall, Cleveland Public Auditorium.

Please Wear Your Badge

SATURDAY, DECEMBER 30TH

- 9:00 A.M. Section A. *General Physiology*. Papers 100-110. Ballroom, Hotel Hollenden.
- Section B. *Embryology*. Papers 111-121. Assembly Room, Hotel Hollenden.
- Section C. *General Morphology and Ecology*. Papers 122-133. Cypress Room, Hotel Hollenden.

Note: The symposium on "Radiobiology" will continue through Saturday forenoon and afternoon with papers devoted largely to the clinical aspects of the problem.

LIST OF TITLES

WEDNESDAY AFTERNOON SESSION, DECEMBER 27TH

2:00 P.M. Ballroom, Hotel Hollenden

Symposium

“Transition from Aquatic to Land Life”

DR. ALFRED S. ROMER, presiding

1. Routes that animals took in emigrating from sea to land. A. S. Pearse, Duke University.
2. The reproductive and developmental problem associated with land colonization. John A. Moore, Columbia University.
3. The role of the kidney in the evolution of land animals from water dwelling ancestors. Roy P. Forster, Dartmouth College.

Business Meeting

The Annual Business Meeting of Section F, A.A.A.S., will be held at 4:30 P.M. in the Ballroom of the Hotel Hollenden immediately following the Symposium. The Annual Business Meeting of the American Society of Zoologists will be held in the same room at 4:40 P.M. immediately following the meeting of Section F.

THURSDAY MORNING SESSION, DECEMBER 28TH

This session will be held in 4 concurrent sections.

Section A. *General Physiology*, 9:00 A.M.

Ballroom, West half, Hotel Hollenden

L. V. HEILBRUNN, presiding

4. Water compartments of a gastropod mollusc. A. W. Martin, University of Washington, and M. J. Huston, University of Alberta. (Lantern; 10 min.)
5. Functional life span of *Arbacia* gametes in methylated xanthines. R. H. Cheney, Brooklyn College. (Lantern; 15 min.)
6. An experimental analysis of the spinning behavior of the *Cecropia* silkworm. W. Van der Kloot and C. M. Williams, Harvard University. (Lantern; 15 min.)
7. Electrical control of growth axis in a regenerating annelid. G. Marsh and H. W. Beams, State University of Iowa. (Lantern; 15 min.)

8. Cuticular and spiracular respiration in *Phormia* larvae. J. B. Buck and M. L. Keister, National Institutes of Health. (Lantern; 15 min.)
9. Mechanism of gas transport through spiracles. John B. Buck and Margaret L. Keister, National Institutes of Health. (Lantern; 15 min.)
10. The effect of varying the NaCl concentration on precipitate formation in the chicken antiserum-antigen system. Morris Goodman, Harold R. Wolfe and Stata Norton, University of Wisconsin. (Lantern; 12 min.)
11. Some sources and characteristics of the heat-labile nutritional requirement(s) of the nematode, *Rhabditis briggsae*. Ellsworth C. Dougherty, University of California (Berkeley) and California Institute of Technology. (Lantern; 15 min.)
12. Retention of injected colloidal thorium dioxide in the body of the laboratory mouse. Thomas W. Easton, Brown University. (Introduced by J. Walter Wilson.) (Lantern; 8 min.)
13. Some effects of sodium azide upon developmental stages of *Stagnicola reflexa*. Arthur L. Schipper, University of Notre Dame. (Lantern; 7 min.)
14. A pharmacological study of the daphnid heart. Sr. M. Aelred Pottinger and Arthur L. Schipper, University of Notre Dame. (Lantern; 7 min.)
15. The effect of anoxia on x-ray-induced injury in *Melanoplus differentialis* embryos. Theodore N. Tahmisian and Dorothy M. Adamson, Argonne National Laboratory. (Lantern; 7½ min.)
16. Oxidase increase in *Melanoplus differentialis* eggs caused by x-irradiation. Theodore N. Tahmisian and Dorothy M. Adamson, Argonne National Laboratory. (Lantern; 7½ min.)

THURSDAY MORNING SESSION, DECEMBER 28TH

Section B. *Embryology*, 9:00 A.M.

Ballroom, East half, Hotel Hollenden

ROBERT S. MCEWEN, presiding

17. Head cavities of the human embryo. Perry W. Gilbert, Cornell University. (Lantern; 15 min.)
18. Differentiation of viscera and survival of tadpoles with opened body cavities. Norman E. Kemp, University of Michigan. (Lantern; 10 min.)
19. Effect of gene dosage on development of pigment pattern in heterozygous triploid axolotls. H. Clark Dalton, Washington Square College, New York University. (Lantern; 15 min.)
20. Induction of regeneration of the limb of the adult frog by augmentation of the nerve supply. Marcus Singer, Harvard Medical School. (Introduced by G. B. Wislocki.) (Lantern; 15 min.)
21. Morphogenetic action of carcinogens in limb regeneration. Alexander G. Karezmar and George G. Berg, Georgetown University Medical Center. (Lantern; 15 min.)

22. Tumor agents as modified differentiated components of cells. S. Meryl Rose and Florence C. Rose, University of Illinois. (Lantern; 15 min.)
23. The effect of flank and gill tissues on the organogenesis of the urodele limb. Charles E. Wilde, Jr., University of Pennsylvania. (Lantern; 15 min.)
24. An analysis of the spatial distribution, tract specificity and orientation of feather germs in the humeral tract of the chick wing. John W. Saunders, Jr., Marquette University. (Lantern; 15 min.)
25. The effects of radiation on the preimplantation stages of the mouse embryo. Liane Brauch Russell and W. L. Russell, Oak Ridge National Laboratory. (Lantern; 15 min.)
26. The expression of a dominant gene in the development of the pigment pattern of *Rana pipiens*. Alice S. Baker, Florida State University. (Introduced by John A. Moore.) (Lantern; 15 min.)
27. Reversal of proximo-distal polarity in limbs of adult newts. J. N. Dent, University of Virginia. (Lantern; 12 min.)

THURSDAY MORNING SESSION, DECEMBER 28TH

Section C. *Endocrinology*, 9:00 A.M.

Assembly Room, Hotel Hollenden

L. V. DOMM, presiding

28. Influence of estrogen on the reproductive system and fertility of 10-day-old mice. J. H. Leatham and R. J. Merklin, Rutgers University. (Lantern; 15 min.)
29. Influence of relaxin on mammary development. Fern A. Garrett and R. V. Talmage, The Rice Institute. (Lantern; 10 min.)
30. B-glucuronidase activity and tissue growth. E. Knobil, Cornell University. (Introduced by H.-B. Adelman.) (Lantern; 10 min.)
31. A study of the uterine B-glucuronidase levels in the rat. S. L. Leonard and E. Knobil, Cornell University. (Lantern; 15 min.)
32. Effect of estrogen on genital phosphatase activities in the male guinea pig. Howard A. Bern, University of California (Berkeley). (Lantern; 8 min.)
33. Influence of adrenal cortical secretion on vitamin A metabolism in the rat. Calvin T. Klopp, M.D., George Washington University School of Medicine. (Introduced by Dr. Warren Andrew.) (Lantern; 10 min.)
34. Reinvestigation of reproductive functions of pituitary stalk-sectioned rats. Roy O. Greep and Russell J. Barrnett, Harvard Medical School. (Lantern; 15 min.)
35. Evidence of panhypopituitarism in pituitary stalk-sectioned rats. Russell J. Barrnett and Roy O. Greep, Harvard Medical School. (Introduced by Helen W. Deane.) (Lantern; 15 min.)
36. Localized metamorphic changes in the skin of *Rana pipiens* larvae by subcutaneous implants of mouse and frog thyroid glands containing radioactive iodine. Jane Couffer Kaltenbach, State University of Iowa. (Introduced by Titus C. Evans.) (Lantern; 15 min.)

37. The antagonistic effect between thyroxin and vitamin A on anuran metamorphosis. Jerome J. Klenner and Joseph F. Gennaro, Jr., University of Pittsburgh. (Lantern; 10 min.)
38. Some effects of phenylalanine and tyrosine deficient diets on the rat. Earl B. Scott, Charles Schwartz and Ralph Ferguson, University of South Dakota, School of Medicine. (Lantern; 15 min.)

THURSDAY MORNING SESSION, DECEMBER 28TH

Section D. *Cytology and Protozoology*, 9:00 A.M.

Cypress Room, Hotel Hollenden

C. W. METZ AND WILLIS H. JOHNSON, presiding

39. Feulgen studies of isolated nuclei following treatment with heparin. Henry S. Roberts, Jr. and Norman G. Anderson, Duke University. (Introduced by George T. Hargitt.) (Lantern; 15 min.)
40. A differential stain for ribonucleic and desoxyribonucleic acids. Martin H. Flax and Marion H. Himes, Columbia University. (Introduced by Arthur W. Pollister.) (Lantern; 15 min.)
41. A cytological study of tumorous growths in a strain of *Drosophila melanogaster*. Ernest W. Hartung, Rhode Island State College. (Lantern; 12 min.)
42. A cytochemical study of oogenesis and cleavage. Max Alfert, University of California. (Introduced by Curt Stern.) (Lantern; 15 min.)
43. Further data on "triple repeats" in *Sciara*. C. W. Metz and J. Paul Reynolds, University of Pennsylvania. (Lantern; 10 min.)
44. The action of aldehyde-coupling reagents on the Feulgen reaction. I. Gelatin-desoxyribonucleate preparations. M. A. Lessler and M. J. Kopac, Washington Square College, New York University. (Lantern; 15 min.)
45. An electron microscope study of mitosis in the onion root tip. Albert W. Sedar and Donald F. Wilson, The State University of Iowa. (Introduced by H. W. Beams.) (Lantern; 10 min.)
46. Electron microscopy of thin-sectioned *Spirostomum*. Harold E. Finley, Howard University. (Lantern; 10 min.)
47. Sexual differentiation in *Rhabdostylia vernalis*. Harold E. Finley and Philip A. Nicholas, Howard University. (Lantern; 10 min.)

THURSDAY AFTERNOON SESSION, DECEMBER 28TH

This session will be held in 3 sections, as shown below

Section A. *Cellular Physiology*, 2:15 P.M.

Room 44, Biology Building, Western Reserve University

WILLIAM H. COLE, presiding

48. Functional studies on molluscan epithelium. E. Ronkin and R. R. Ronkin, University of Delaware. (Introduced by Secretary.) (Lantern; 15 min.)

49. Experiments on the physiology of the rumen. R. E. Hungate, Washington State College, and R. W. Dougherty, Cornell University. (Lantern; 15 min.)
50. The effect of *Clostridium perfringens* α -toxin on the osmotic behavior of chicken erythrocytes. F. R. Hunter and Jane A. Bullock, Florida State University. (Lantern; 8 min.)
51. Effect of various inhibitors on the respiration of *Paramecium caudatum*. Elaine Nelson and Keatha K. Krueger, University of South Dakota. (Introduced by W. L. Hard.) (Lantern; 10 min.)
52. The nucleic acids of the larval salivary gland of *Drosophila robusta* and the possible role of the nucleolus. S. W. Leshner, University of Kansas. (Introduced by Hampton L. Carson.) (Lantern; 10 min.)
53. Anti-mitotic substances in living tissues. L. V. Heilbrunn and John W. Kelly, University of Pennsylvania. (Lantern; 15 min.)
54. Retardation throughout the mitotic cycle of dividing sea-urchin eggs by the "prophase poisons," urethans and nitrogen mustards. Ivor Cornman, George Washington University. (Lantern; 12 min.)
55. The site of action of ethyl carbamate and sodium azide in the living embryonic cell. Joseph Hall Bodine and Kiao-Hung Lu, State University of Iowa. (Lantern; 15 min.)
56. The respiration of isolated blastomeres and polar lobes of the eggs of *Mytilus edulis*. William E. Berg and Phyllis B. Kutsky, University of California (Berkeley). (Introduced by R. M. Eakin.) (Lantern; 15 min.)
57. Effect of radiation from intraperitoneally injected radium chloride upon megakaryocyte and blood platelet production in albino rats. Paul G. Roope, Hal Bingham, Ralph Comer and Martha Madison, University of Kansas. (Lantern; 15 min.)

THURSDAY AFTERNOON SESSION, DECEMBER 28TH

Section B. *General Demonstrations*, 2:15 P.M.

Room 32, Biology Building, Western Reserve University

Joint session with the American Society of Parasitologists

A. H. HEESE, presiding

58. Larval lampreys from the Champlain area. Francis H. Wilson, Champlain College.
59. Comparison of transplantability of skin, ovary and tumor in mice. Meredith N. Runner and Joy Palm, Jackson Memorial Laboratory.
60. The Golgi apparatus as revealed in desilvered Da Fano preparations. F. B. Adamstone, University of Illinois.
61. Pigment-gene pleiotropy in the Caribe-Cuna Moon-Child. Clyde E. Keeler, Georgia State College for Women. (Introduced by Secretary.) (Lantern.)
62. Staining of nucleic acids by azure-phthalate. Marion H. Himes and Martin H. Flax, Columbia University. (Introduced by Arthur W. Pollister.)

63. Ultrasonic sounds of bats, their acoustical dimensions and biological significance. Donald R. Griffin, Cornell University.
64. Electron micrographs of thin-sectioned Spirostomum. Harold E. Finley, Howard University.
65. Electron micrographs of dividing cells. H. W. Beams and T. C. Evans, The State University of Iowa.
66. Multiple pores and contractile vacuoles in Paramecium. R. L. King, The State University of Iowa.
67. The acquisition of thyroxine-sensitivity by tadpole tissues. William Etkin, The City College of New York.
68. Electron micrographs of connective tissue spread preparations showing the submicroscopic network of fibrils. F. Wassermann, Argonne National Laboratory.
69. Regression in denervated injured limbs of Amblystoma. C. S. Thornton and David Kraemer, Kenyon College.
70. Cystic lungs, a congenital abnormality associated with the short ear gene in the house mouse. Jean Smith, Ohio State University. (Introduced by E. L. Green.)
71. The development in vitro of giant cells from human peripheral blood. M. Goldstein, Western Reserve University.
72. The origin and development of the respiratory labyrinth in the paradise fish *Macropodus opercularis*. S. Ito, Western Reserve University.
73. The significance of mitochondria in embryonic chick pancreas. R. D. Shieman, Western Reserve University.

Note: For a list of demonstrations by The American Society of Parasitologists see the A.A.A.S. General Program.

THURSDAY AFTERNOON SESSION, DECEMBER 28TH

Section C. *Demonstrations by Motion Pictures*

Auditorium of the Cleveland Academy of Medicine, 2009 Adelbert Road

(near Biology Building, Western Reserve University)

RALPH BUCHSBAUM, presiding

74. Resumption of heart-beat in chick embryos frozen in liquid nitrogen. B. J. Luyet and F. Gonzales, St. Louis University. (12 min.)
75. Survival of vinegar "eels" after congelation in liquid nitrogen. B. J. Luyet and P. M. Gehenio, St. Louis University and Biodynamica Research Laboratory. (12 min.)
76. White thrombo-embolism in the transilluminated cheek pouch of the hamster following trauma, anti-coagulant therapy, infection and malignant neoplasia. B. R. Lutz, G. P. Fulton and R. P. Akers, Boston University. (15 min.)
77. Serial sarcoma transplantation in the hamster cheek pouch, and the effect of advanced neoplasia on the small blood vessels. B. R. Lutz, G. P. Fulton, D. I. Patt, A. H. Handler and D. F. Stevens, Boston University. (15 min.)

78. Preservation of red cells in blood frozen in liquid nitrogen. B. J. Luyet and L. Menz, St. Louis University. (10 min.)
79. Intrinsic contractility in the embryonic rat heart. E. K. Hall, University of Louisville School of Medicine.
80. Life cycle of *Diphylobothrium latum* (broad fish tapeworm). M. S. Ferguson, U. S. Public Health Service. (30 min.)

FRIDAY FORENOON SESSION, DECEMBER 29TH

This session will be held in three concurrent sections

Section A. *Animal Biology and Sociobiology*, 9:00 A.M.

Ballroom, Hotel Hollenden

J. P. SCOTT, presiding

81. Social behavior and organization in the dog. J. P. Scott and John L. Fuller, Roscoe B. Jackson Memorial Laboratory. (Moving pictures; 15 min.)
82. The effect of X irradiation on fighting behavior in male mice. Howard H. Vogel, Jr., Argonne National Laboratory. (Lantern; 16 mm motion picture; 15 min.)
83. Hereditary differences in social behavior and social organization between dba and C57 black inbred strains of the house mouse (*Mus musculus*). John B. Calhoun, Roscoe B. Jackson Memorial Laboratory. (Lantern; 15 min.)
84. Age and strain differences in response to electro-convulsive-stimulation (ECS) in the mouse. Alfred Coulombre, Roscoe B. Jackson Memorial Laboratory. (Introduced by J. L. Fuller.) (Motion picture; 15 min.)
85. The level of thyroid activity in the guinea pig. Mina MacNair Brown, Roy R. Peterson, Barbara Rayner and William C. Young, University of Kansas. (Lantern; 7 min.)
86. Sex drive and the level of thyroid activity in the male guinea pig. William C. Young, Roy R. Peterson, Barbara Rayner and Mina MacNair Brown, University of Kansas. (Lantern; 8 min.)
87. Sexual behavior in the guppy. Eugenie Clark and Lester R. Aronson, American Museum of Natural History. (Lantern; 15 min.)
88. Factors influencing embryonic survival outside of the mouth of the oral incubating fish, *Tilapia macrocephala*. Evelyn S. Shaw, American Museum of Natural History, New York University and Newark College, Rutgers University. (Introduced by C. M. Breder, Jr.) (Lantern; 15 min.)
89. Attentive behavior of the house wren, *Troglodytes aedon*. S. Charles Kendeigh, University of Illinois. (Lantern; 15 min.)
90. Territorial and social behavior in nesting cliff swallows. John T. Emlen, Jr., University of Wisconsin. (Lantern; 15 min.)
91. Additional observations on the sage grouse. John W. Scott, University of Wyoming. (16 mm motion pictures; 15 min.)

92. Some basic psychological and neural mechanisms of social behavior in chicks. N. E. Collias, University of Wisconsin. (Lantern; 8 min.)
93. The socialization of chicks. N. E. Collias, University of Wisconsin. (Lantern; 7 min.)

FRIDAY FORENOON SESSION, DECEMBER 29TH

Section B. *Endocrinology*, 9:00 A.M.

Assembly Room, Hotel Hollenden

F. L. HISAW, presiding

94. Further evidence of facilitation of comb response to androgen by thyroid hormone. David M. Morris, North Texas State College. (Lantern; 15 min.)
95. The effect of mammalian thyroid powder and thiouracil on growth rates and on the differentiation of the gonopod in *Lebistes reticulatus*. Arthur F. Hopper, Rutgers University. (Lantern; 15 min.)
96. Biological activity of 17-vinyl testosterone. C. H. Howell and N. W. Antonchak, Ciba Pharmaceutical Products. (Introduced by L. A. Stauber.) (Lantern; 15 min.)
97. The inhibition of uterine growth by a sulfhydryl inhibitor. H. A. Salhanick, Melvin H. Farmelant, Thomas C. Smith and Frederick L. Hisaw, Harvard University. (Lantern; 15 min.)
98. A comparison of reproductive potential of two rat populations. David E. Davis. The Johns Hopkins School of Hygiene and Public Health. (Lantern; 10 min.)
99. Progesterone levels in the ewe. G. M. Neher and M. X. Zarrow, Purdue University. (Lantern; 10 min.)

FRIDAY FORENOON SESSION, DECEMBER 29TH

Ballroom, Public Auditorium, 9:30 A.M.

Section C. Symposium on "Radiobiology"

Joint session with Section Nm — Medicine

J. H. BODINE, presiding

1. The effects of low intensities of radiation on plant development. Kark Sax.
2. Plant growth and growth hormone relationships as affected by ionizing radiation. Solon A. Gordon.
3. Radiation-induced mutations in chemical requirements of *Salmonella*. H. H. Plough.
4. Modification of the frequency of radiation-induced chromosome changes and mutations and their significance. Carl P. Swanson.
5. The genetic effects of radiation in mammals. W. L. Russell.

FRIDAY AFTERNOON SESSION, DECEMBER 29TH

Two concurrent symposia

Symposium on "*Genetics and Behavior*," 2:00 P.M.

Ballroom, Hotel Hollenden

Session arranged by A.A.A.S.

CALVIN S. HALL, presiding

1. Factors in maternal behavior and early socialization. Dr. T. C. Schneirla, The American Museum of Natural History.
2. Genetics, temperament and social behavior. Dr. J. P. Scott, R. B. Jackson Memorial Laboratory.
3. Gene mechanisms and behavior patterns. Dr. John L. Fuller, R. B. Jackson Memorial Laboratory.

Symposium on "*Radiobiology*," 2:00 P.M.

Ballroom, Public Auditorium

Joint session with Section Nm—Medicine

H. H. PLOUGH, presiding

1. The effects of radiation on some chemical compounds. A. K. Solomon.
2. The radiation chemistry of cysteine solutions. S. L. Whiteher and M. Rotheram.
3. Some experiments on the fundamental action of x-rays on cells. Titus C. Evans.
4. Secondary factors influencing the sensitivity of living cells to radiation. Alexander Hollaender.
5. The effects of local x-ray irradiation upon the development of jaws in the young axolotl, *Siredon mexicanum*. V. V. Brunst, E. A. Sheremetieva-Brunst and F. H. J. Figge.
6. Effects of radioactive iodine on the rat thyroid. Simon Koletsky.

SATURDAY FORENOON SESSION, DECEMBER 30TH

This session will be held in 3 concurrent sections

Section A. *General Physiology*, 9:00 A.M.

Ballroom, Hotel Hollenden

J. H. BODINE, presiding

100. Respiratory movements, oxygen consumption and brain metabolism in temperature acclimatization of goldfish. John A. Freeman, Newcomb College of Tulane University. (Lantern; 15 min.)
101. An improved method for the artificial insemination of mice. J. C. Kile, Jr., Oak Ridge National Laboratory. (Introduced by William L. Russell.) (Lantern; 15 min.)

102. The respiration of the *Cecropia* silkworm in the presence of high pressures of carbon monoxide. Howard A. Schneiderman, Ned Feder and Carroll M. Williams, Harvard University. (Introduced by H. B. Bigelow.) (Lantern; 15 min.)
103. Oxidative enzymes in relation to pupal diapause and adult development in the *Cecropia* silkworm. Richard C. Sanborn and Carroll M. Williams, University of Illinois and Harvard University. (Introduced by A. B. Dawson.) (Lantern; 15 min.)
104. The moulting-fluid of the *Cecropia* silkworm. Janet V. Passonneau and Carroll M. Williams. Argonne National Laboratory and Harvard University. (Introduced by G. H. Parker.) (Lantern; 15 min.)
105. An immunological study of the larval-pupal transformation of the *Cecropia* silkworm. William H. Telfer and Carroll M. Williams, Harvard University. (Introduced by A. S. Romer.) (Lantern; 15 min.)
106. Implication of aerobic phosphorylation in the active cellular transport of phenol red by isolated renal tubules. R. P. Forster, Dartmouth College, and J. V. Taggart, Columbia University. (Lantern; 15 min.)
107. Dependence of ciliary action on acetylcholine esterase activity. Gerald R. Seaman, University of Texas, Medical Branch. (Lantern; 15 min.)
108. Parallel action of drugs on animals and on electrical phase-boundary potentials *in vitro*. T. C. Barnes and R. Beutner, Hahnemann Medical College and Medical College of Alabama. (Lantern; 15 min.)
109. Individual specificity in the fusion of hydroid stolons and the relationship between stolon growth and colony form. Sears Crowell, Indiana University. (Lantern; 15 min.)
110. Preliminary study of the hemoconcentration response of albino rats to acute hypothermia. A. C. Higginbotham and M. R. Siers, Florida State University. (Introduced by F. R. Hunter.) (Lantern; 7 min.)

SATURDAY FORENOON SESSION, DECEMBER 30TH

Section B. *Embryology*, 9:00 A.M.

Assembly Room, Hotel Hollenden

D. E. MINNICH, presiding

111. Early embryology of the dog. H. T. Gier, Kansas State College. (Lantern; 8 min.)
112. Placentation in the dog. D. Romanucci, Kansas State College. (Introduced by H. T. Gier.) (Lantern; 7 min.)
113. Simultaneous anterior and posterior regeneration and other growth phenomena in *Clymenella* and related polychaetes. Gairdner B. Moment, Goucher College. (15 min.)
114. The accumulation of phosphatase in the duodenum of mouse embryos and young mice. Florence Moog, Washington University. (Lantern; 12 min.)

115. Effects of ethyl carbamate (urethane) on the early development of the teleost, *Brachydanio rerio*. Kenneth Hisaoka, University of Western Ontario. (Introduced by Helen I. Battle.) (Lantern; 15 min.)
116. The modification of developmental pattern in the sand dollar with sodium selenite. Olin Rulon, Northwestern University. (Lantern; 15 min.)
117. Effects of thiourea, thiouracil, phenylthiourea, and propylthiouracil on mortality, growth, and differentiation of *Drosophila melanogaster*. Morris H. Harnly and E. D. Goldsmith, New York University. (Lantern; 15 min.)
118. The growth of mesencephalic V nucleus cells as a metamorphic event in anurans. Jerry J. Kollros, Virginia Pepernik, Robert Hill and Jane C. Kaltenbach, The State University of Iowa. (Lantern; 15 min.)
119. Differentiation by loss of biochemical synthetic ability. Clement L. Markert, University of Michigan. (Lantern; 15 min.)
120. Carbohydrate metabolism in insulin-treated chick embryos. Edgar Zwilling, University of Connecticut. (Lantern; 10 min.)
121. Ectrodactylism (split hand) inherited as a dominant with low penetrance. Charles D. Howell, Muskingum College. (Lantern; 15 min.)

SATURDAY FORENOON SESSION, DECEMBER 30TH

Section C. *General Morphology and Ecology*, 9:00 A.M.

Cypress Room, Hotel Hollenden

A. S. PEARSE AND B. R. COONFIELD, presiding

122. The local effect of vitamin A on rat epidermis. Joseph Sabella, Howard A. Bern and Raymond H. Kahn, University of California (Berkeley). (Lantern; 7 min.)
123. Metabolic types and growth types. Ludwig von Bertalanffy, University of Ottawa. (Introduced by Daniel P. Quirring.) (Lantern; 15 min.)
124. Vascular, neural, and skeletal anomalies associated with the short ear gene in the mouse. Margaret C. Green, Ohio State University. (Introduced by C. E. Venard.) (Lantern; 10 min.)
125. The gill area of marine fishes — a comparative study. I. E. Gray, Duke University. (Lantern; 15 min.)
126. A threshold concept for interpreting variations in the axial skeleton of inbred strains of mice. E. L. Green, Ohio State University. (Lantern; 15 min.)
127. Studies on nutritional anemia in *Triturus viridescens*. Sophie Jakowska and Ross F. Nigrelli, College of Mt. St. Vincent and New York Zoological Society. (Lantern; 15 min.)
128. Structure of the vertebrate liver. Hans Elias, The Chicago Medical School. (Lantern; 15 min.)
129. The red and black pigments of *Plethodon cinereus*. Emanuel C. Hertzler, Kent State University. (Introduced by Frederick H. Test.) (Lantern; 10 min.)

130. Role of food habits in interspecific competition between Michigan garter snakes. Charles C. Carpenter, University of Michigan. (Introduced by F. H. Test.) (Lantern; 15 min.)
131. Response of a population of wild house mice (*Mus musculus*) to restricted food supply. Robert L. Strecker and John T. Emlen, Jr., University of Wisconsin. (Lantern; 10 min.)
132. Aerial observations of homing pigeons. Harold B. Hitchcock, Middlebury College. (Lantern; 12 min.)
133. A factorial study in the systematics of *Kaloterpes*. Clyde P. Stroud, University of Chicago. (Introduced by A. E. Emerson.) (15 min.)

ABSTRACTS

TABLE OF CONTENTS

Papers Presented in Person

<i>Sections</i>	<i>Abstract No.</i>	<i>Page</i>
Presidential Symposium	1-3	509-510
General Physiology	Section A 4-16	510-516
Embryology	Section B 17-27	517-522
Endocrinology	Section C 28-38	522-528
Cytology and Protozoology	Section D 39-47	528-533
Cellular Physiology	Section A 48-57	533-537
General Demonstrations	Section B 58-73	537-543
Demonstrations by Motion Pictures	Section C 74-80	543-546
Animal Behavior and Sociobiology	Section A 81-93	547-553
Endocrinology	Section B 94-99	553-556
General Physiology	Section A 100-110	556-561
Embryology	Section B 111-121	561-566
General Morphology and Ecology	Section C 122-133	567-572

Papers Presented by Title

Cellular Physiology	134-141	572-576
Cytology	142-146	576-579
Ecology	147-149	579-580
Embryology	150-167	581-589
Endocrinology	168-189	589-601
General Morphology	190-195	601-604
General Physiology	196-234	604-623
Genetics	235-236	623-624
Histology	237	624-625
Parasitology	238-240	625-626
Protozoology	241	627

ABSTRACTS

PAPERS PRESENTED IN PERSON

Presidential Symposium

1. ROUTES FROM SEA TO LAND. A. S. Pearse, Duke University. (Lantern; 40 min.)

Animals on marine beaches mostly have developmental stages that require an aquatic environment and most of them are sessile and therefore cannot become permanently established on land. Some are held in definite zones by marine predators. A few types (*Birgus*, *Coenobita*, etc.) that have developed ability to carry on respiration in air and have adaptations that prevent them from drying up have successfully invaded land habitats and return to the ocean only to leave their young. Estuaries are often silty and lack oxygen. In them certain aquatic animals have developed ability to breathe air and to some extent take advantage of the food resources and comparative safety of land habitats. Some animals are afraid of the predators that come in with the tide. Fiddler crabs commonly retreat into their burrows and close up the entrance when the tide rises. Some swamp snails lay their eggs above the water where there is abundant oxygen and aquatic predators will not devour them. Pulmonate snails are common in swamps. The water in swamps and marshes varies in amount and animals that live in such habitats are burrowers or have exoskeletons that prevent desiccation. Such adaptations have enabled a few of them to attain to life on land. In Siam there is a fish (*Ophiocephalus*) that lives for four months without water, buried in dry rice fields; and there the climbing perch (*Anabas*) wanders over the land catching insects.

2. THE REPRODUCTIVE AND DEVELOPMENTAL PROBLEMS ASSOCIATED WITH LAND COLONIZATION. John A. Moore, Departments of Zoology, Barnard College and Columbia University.

Water, which is the most abundant protoplasmic compound, is also the most abundant environmental compound for most classes of the animal kingdom. Members of only a few classes are able to carry out their entire life cycle on dry land. Drastic physiological and morphological changes have occurred during the transition from water to land but the present report will be restricted to a consideration of the problems associated with reproduction and embryonic development. Comparisons of present day fish, amphibia, and reptiles suggest, in broad outline, the problem and solution. Living amphibia provide some hints as to details.

3. THE ROLE OF THE KIDNEY IN THE EVOLUTION OF LAND ANIMALS FROM WATER DWELLING ANCESTORS. Roy P. Forster, Dartmouth College.

During the course of evolution the migration of animals from aquatic to terrestrial environments has been dependent in great part upon renal adaptations resulting in the conservation of water and in the efficient elimination of potentially toxic compounds arising from the deamination of α amino acids. Despite drastic changes in the external environment which accompanied these migrations the body fluids making up the internal environment remained relatively unaffected. This constancy of the kidney's real environment obviated for this organ the progressive series of adaptive structural variations noted with limbs, the body surface, respiratory devices and those parts in direct contact with the changing external environment. However, certain specializations in excretory organs are related to specific habitats and these are summarized, particularly those concerning alterations in glomerular or filtering activity and in renal tubular transport.

Identical excretory functions are carried out in various animals by a diversity of structures having widely different embryological origins. Hence, nephridia, coelomoducts, nephromixia and Malpighian tubules in Invertebrates are physiologically indistinguishable, and almost identical activities are carried on in the pro-, meso- and metanephros of Vertebrates.

With some confidence one can deduce those adaptations animals must have made in the course of their evolutionary migration from aquatic to terrestrial environments from observations made on the comparative anatomy and physiology of surviving forms. Such studies on the kidney are not meaningful unless concomitant alterations in functions carried out by the gut, respiratory devices and the body surface are also considered.

General Physiology

Section A

4. WATER COMPARTMENTS OF A GASTROPOD MOLLUSC. Arthur W. Martin, University of Washington, and Mervyn J. Huston, University of Alberta.¹

Certain invertebrate animals possess a large amount of organically dilute blood. The circulatory physiology of these animals may prove of interest. We therefore sought means of estimating the sizes of the standard fluid compartments: vascular, extracellular and intracellular.

In the gastropod *Tethys californicus* such standard blood volume indicators as vital red and T 1824 proved of no value since they stained the tissues. For determination of the blood volume substances of high molecular weight or dispersed in larger than molecular units seemed essential. Hemoglobin, homogenized cream and mild silver protein were all tested and yielded results which did not differ significantly each from the others. The average space found with hemoglobin was 75.6 per cent of the body weight with a range of 63.9–84.5. The average space occupied by cream was 70.5%, range 60–87.4. The average space occupied by mild silver protein was 75.2 per cent, range 59.7–86.1. The space so determined is presumed to be the vascular space.

The insulin space, generally accepted as the extracellular compartment, was determined by standard methods to average 83.3 per cent of the body weight with a range of 72.4–90.8. This value is significantly larger than those for the vascular space. Finally the total water was determined by desiccation at 105°C. to average 94.4 per cent of the body weight with a range of 92.6–95.8. Thus, by difference, each of the compartments is known.

¹ Supported in part by a grant from the Office of Naval Research.

5. FUNCTIONAL LIFE SPAN OF ARBACIA GAMETES IN METHYLATED XANTHINES. Ralph Holt Cheney, Brooklyn College and the Marine Biological Laboratory, Woods Hole. (Lantern; 15 min.)

The author has demonstrated (Bio. Bull., October, 1950) that the functionally normal, post-shedded life span of *Arbacia* sperm and eggs have a relative survival ratio of 2:1. Recommendation was made that eggs not over eight hours, and sperm not over 24 hours, be employed for experimentation. In many instances, however, eggs were fertilizable up to 24 hours and sperm often retained fertilizing capacity for 48 hours.

Arbacia gametes were shed into di- and trimethylated xanthines, mixed, and observed for their fertilizability or fertilizing power under conditions identical with previous studies except that media employed were different concentrations of theophylline, theobromine, or caffeine, in sea water. M/400 concentrations (maximal for theobromine, least soluble of the three alkaloids) of all three drugs were employed. Theophylline effects were observed also in M/200 and M/40; and, caffeine in M/200, M/40, M/10.

In M/400 concentrations of theophylline, theobromine, and caffeine, gametes survived with retention of functional potentialities. Mixing gametes in sea water after various intervals of exposure to drugs up to 18 hours pretreatment, resulted in the formation of fertilization membranes. Development in sea water proceeded. Sperm, after 24 hours in M/400 concentration of any of the drugs still fertilized fresh eggs. Twenty-four hour treated eggs were fertilizable in a higher percentage than non-treated eggs of the same age. Pretreatment of both gametes separately for 24 hours retarded appearance of fertilization membranes but development did occur in sea water. M/200 and M/40 theophylline and caffeine; and, M/10 caffeine all shortened the life span.

6. AN EXPERIMENTAL ANALYSIS OF THE SPINNING BEHAVIOR OF THE CECROPIA SILKWORM. William Van der Kloot and Carroll M. Williams,¹ Harvard University. (Lantern; 15 min.)

In the process of spinning its complex silken cocoon, the mature *Cecropia* silkworm exhibits a remarkable series of instinctive activities. First it spins a compact outer envelope equipped with a valve, then a spongy intermediate layer, and finally an inner compact envelope equipped with a valve. We have used this program of predictable behavior in an experimental study of instinct. Our results demonstrate: (1) The instinct presiding over cocoon formation is present and intact throughout larval life, but, in the immature larvae, is held under bio-

chemical restraint by a hormone secreted by the corpora allata. (2) The program of instinctive activities is so organized within the central nervous system of the insect that no stage in the sequence can be omitted and no stage repeated. (3) The timing of successive events is enforced by an intrinsic mechanism within the insect itself and is essentially independent of environmental cues. (4) The spinning behavior is totally eliminated after even minor surgical injury to the brain. (5) Critical exposure of the mature larvae to carbon dioxide gas serves to eliminate the instinct concerning cocoon formation without interfering with subsequent pupation or adult development. (6) After treatment with carbon dioxide, the spinning instinct behaves in a unitary manner, being either totally lost or fully preserved.

¹ This study was aided by the Lalor Foundation.

7. ELECTRICAL CONTROL OF GROWTH AXIS IN A REGENERATING ANNELID.¹ Gordon Marsh and H. W. Beams, State University of Iowa, Department of Zoology. (Lantern; 15 min.)

Regenerating 6-12 somite section of *Aulophorus furcatus*, imbedded in agar, were exposed to direct current 4-5 days. Aerated medium of controlled specific resistance flowed continuously through the chamber at 20° or 25°C. Variation of specific resistance between 2000 and 20,000 ohm centimeters showed the morphological effects to be due to the potential fall rather than to the current density.

Anodally orientated pieces developed normally below 100 mv/mm, showed retarded tail development (sometimes permanent) and occasional retarded head development at 150-200 mv/mm, bipolarity at 250-300 mv/mm and death above this level. Reversal of axis did not occur. The bipolar individuals died before achieving complete head symmetry on both ends. Cathodally oriented pieces developed normally save at the higher range. Development of temporary functional head tendencies in the tail preceded morphological modification. Interpretation of results and precise determination of field strengths was hindered by frequent turning of the pieces to the cathode and by degeneration of terminal segments at varying times. No regeneration of new segments occurred during exposure.

Mortality was high, and showed no consistent relation to field strength (save near the upper level), specific resistance and composition of medium, oxygen tension between 0.2 and 1.0 atmospheres, CO₂ tension between 0 and 5 per cent, and bath temperature between 20 and 25°C.

¹ Supported by a grant from the American Cancer Society through the NRC Committee on Growth.

8. CUTICULAR AND SPIRACULAR RESPIRATION IN PHORMIA LARVAE. John B. Buck and Margaret L. Keister, Experimental Biology and Medicine Institute, National Institutes of Health. (Lantern; 15 min.)

Cuticular oxygen uptake from air, in larvae with all four spiracles ligated, is about 11% of total normal control uptake. In either ligated or unligated larvae under aerated water it is about 6%.

Uptake from air in larvae with the anterior pair of spiracles alone ligated is identical with that of controls, indicating that the anterior spiracles are unnecessary for normal respiratory needs. Uptake in larvae with only the posterior spiracles closed is about 55% of normal, indicating that the anterior spiracles are patent, though able to pass less gas than the posteriors. Confirmatory evidence is: (1) Much more pressure is required to force gas out of the anterior than posterior spiracles; (2) Low-viscosity oils enter the posterior spiracles preferentially; (3) Larvae in osmic vapor blacken first posteriorly; (4) Larvae with anterior spiracles alone ligated darken evenly and rapidly at pupation, whereas those with posteriors alone ligated darken slowly and unevenly from the front.

In oxygen, uptake is normal in controls (showing absence of oxygen limitation or stimulation) and in larvae with posterior spiracles ligated (showing adequacy of anterior spiracles in raised oxygen). Cuticular respiration of completely ligated larvae increases $3-4 \times$ both in gaseous oxygen and under oxygenated water. None of the uptake of submerged larvae takes place across the spiracular interface.

Normal spiracular apertures are adequate for about 250% of normal oxygen uptake (in DDT-stimulated larvae in air), but in oxygen the uptake of poisoned larvae rises to about 400% of normal.

9. MECHANISM OF GAS TRANSPORT THROUGH SPIRACLES. John B. Buck and Margaret L. Keister, Experimental Biology and Medicine Institute, National Institutes of Health. (Lantern; 15 min.)

Phormia larvae in air take up 11% of their oxygen through the cuticle, the rest via a pair of anterior and a pair of posterior spiracles. With the anterior spiracles ligated the animal approximates a cylinder of protoplasm supplied with oxygen through a single aperture at one end (posterior spiracles). Assuming that (1) tissue PO_2 is zero, (2) all the respiration is concentrated in a single point midway in the body, and (3) free diffusion prevails, the calculated (Fick's law) maximum rate at which oxygen could enter from air through the spiracular openings at presumed maximum dilation is about 10% of the observed, showing that diffusion alone cannot supply enough oxygen for normal respiration in air.

Though ligated larvae move, they show nothing resembling ventilatory pumping. The hypothesis is advanced that gas enters spiracles, as it does leaf stomata (Brown and Escombe, Verduin), in spheroidal diffusion shells such that the amount transported is proportional to some linear dimension of the aperture rather than area. Compatible with this idea: (1) the total apertural area of the posterior spiracles is only 1/60 that of the associated tracheae, whereas the respective perimeters are nearly equal (2:3); (2) the ratio of total tracheal perimeter at the anterior spiracles to that at the posterior is of the same order of magnitude as the estimated ratio of the oxygen uptakes from air through the respective spiracles in larvae in which the metabolism is maximally stimulated by DDT.

10. THE EFFECT OF VARYING THE NaCl CONCENTRATION ON PRECIPITATE FORMATION IN THE CHICKEN ANTISERUM-ANTIGEN SYSTEM. Morris Goodman, Harold R. Wolfe and Stata Norton, University of Wisconsin. (Lantern; 12 min.)

Quantitative (antibody N) and semiquantitative (turbidimetric measurements) studies were made to show the effect of varying concentrations of NaCl on the chicken antiserum-antigen system. Crystalline beef albumin and human gamma globulin were used as antigens. Data compared with mammalian antiserum-antigen systems show very different results. Each increase of NaCl concentration up to that range where salting out of some chicken serum proteins began (14-18%) resulted in a marked increase of precipitation in the antigen excess and apparent equivalence zones, but not in the antibody excess zone. Fractionation of antisera showed that only the antibody containing fraction (water insoluble globulin) was concerned. The amounts of antibody N and antigen N precipitated in the high salt concentrations were much greater than in the low salt concentrations, but the ratios of antibody N to antigen N in the maximal precipitate were not greater. This demonstrated the specificity of the increased precipitation. Not only was there incompleteness of antibody precipitation in low salt concentration, but the data also indicated that the speed of the reaction was much slower. It was concluded that to perform accurate quantitative studies on chicken precipitin-serum protein systems a NaCl concentration of about 8% should be used.

11. SOME SOURCES AND CHARACTERISTICS OF THE HEAT-LABILE NUTRITIONAL REQUIREMENT(S) OF THE NEMATODE, *RHABDITIS BRIGGSÆ*. Ellsworth C. Dougherty,¹ University of California (Berkeley) and California Institute of Technology. (Lantern; 15 min.)

It has previously been shown that *R. briggsæ* can be grown axenically on fresh chick embryo juice (CEJ) supplemented with the Kidder-Dewey *Tetrahymena* (basal) medium. Other sources of the heat-labile components(s) of CEJ essential for *R. briggsæ* have subsequently been sought. The following have been shown to possess activity, when added to basal medium, permitting the maturation and usually the reproduction of the nematode: (1) lyophilized commercial CEJ (Difco); (2) aqueous liver extract (LE) prepared by high speed centrifugation and sterilized by a procedure involving the addition of toluene followed by lyophilization; and (3) human whole blood and plasma. Of these, blood and plasma permit maturation, but the few II generation larvae produced do not grow. The active component or components in CEJ and LE have been found not to pass through a semi-permeable membrane (Visking dialyzing tubing). Studies on LE have demonstrated that activity follows the protein fraction precipitated at 0.75 saturation with pH 7.0-buffered $(\text{NH}_4)_2\text{SO}_4$, filtered (or centrifuged at high speed), suspended in buffer, and reprecipitated; no activity has yet been found in the unprecipitated portion left after the first precipitation and a subsequent high-speed centrifugation. At least one heat-labile requirement thus has been shown to behave like protein; until this one or more protein-like factors are precisely characterized, they are hereby collectively designated "factor Rb."

¹ Research Fellow of the American Cancer Society as recommended by the Committee on Growth of the National Research Council, 1949-52.

12. RETENTION OF INJECTED COLLOIDAL THORIUM DIOXIDE IN THE BODY OF THE LABORATORY MOUSE. Thomas W. Easton, Brown University. (Introduced by J. Walter Wilson.) (Lantern; 8 min.)

Mice were injected intravenously through the tail vein with colloidal ThO_2 (Thorotrast), 0.2 cc per day on alternate days for three doses, a total of 0.6 cc. Success of injections was checked by roentgenogram, a heavy shadow at base of tail indicating poor injection. Such mice were not used. Thorotrast in the body was determined by ashing the entire mouse after dividing into various samples, and measuring the radioactivity with a Geiger counter. Thorotrast was determined in the whole body, in liver and spleen alone, and in the rest of the body by comparing measured activity with that of measured amounts of the material injected.

Values obtained were as follows: one day after last injection, mice retained from 0.4 to 0.59 cc of the original 0.6 cc. Of this the liver represented 25–55%; the spleen, 14–28%; and the remainder of the body, 17–51%. Ten days after last injection the total was 0.4 cc, with variations up to ± 0.1 cc. Of this the liver held 55–70%; the spleen, 25–30%; and the rest of the body, 5–20%. This level was maintained for 130 days, at least.

Most of the variations observed in amount of Thorotrast retained appear to be due to variations in liver content. Neither splenectomy nor fasting begun prior to injection appear to have any marked effect on retention or loss of ThO_2 .

13. SOME EFFECTS OF SODIUM AZIDE UPON DEVELOPMENTAL STAGES OF *STAGNICOLA REFLEXA*. Arthur L. Schipper, University of Notre Dame. (Lantern; 7 min.)

Clutches of eggs of *Stagnicola reflexa*, representing various developmental stages, were placed in 0.154 M, 0.0154 M, and 0.00154 M solutions of sodium azide for periods ranging from fifteen minutes to two hours. After rinsing the eggs were returned to tap water. Such treatment resulted in:

- (1) an extension of the developmental period;
- (2) a decrease
 - (a) in the number of embryos developing;
 - (b) in the per cent hatching;
 - (c) in the heart beat/minute;
- (3) a reduction in the size of the embryos;
- (4) an increase in the number of embryos malformed.

There was a pronounced resistance uncleaved egg to the effects of sodium azide whereas the early cleavage stages were very susceptible. An increasing resistance was observed with the appearance of motile embryos.

14. A PHARMACOLOGICAL STUDY OF THE DAPHNID HEART. Sr. M. Aelred Pottinger and Arthur L. Schipper, University of Notre Dame. (Lantern; 7 min.)

A study of drug action on the heart of *Daphnia magna* was made to determine the myogenicity or neurogenicity, as well as the mechanism of neurohumoral control.

The following drugs were used: quinidine, alpha-fagarine, acetylcholine, eserine, atropine, di-isopropyl fluorophosphate, amphetamine (beta-phenyl-isopropylamine),

dibenamine (dinitro-dibenzyl-beta-chlorethylamine hydrochloride), and ergotamine tartrate (gynergen).

The results obtained bear evidence of the neurogenicity of the daphnid heart and of the cholinergic mediation of nervous stimuli. The assumption that the heart of Branchiopoda, alone among the Crustacea thus far studied, has mutated to myogenicity appears unjustifiable in the light of the present investigation.

15. THE EFFECT OF ANOXIA ON X-RAY-INDUCED INJURY IN *MELANOPLUS DIFFERENTIALIS* EMBRYOS. Theodore N. Tahmisian and Dorothy M. Adamson, Argonne National Laboratory. (Lantern; $7\frac{1}{2}$ min.)

Melanoplus differentialis embryos placed in an atmosphere of $N_2/CO_2/95\%/5\%$ for 24 hr developed normally. If these anoxic embryos are irradiated at 25,000 r after 24 hr of anoxia and placed at 25°C. in air negative growth does not occur as with eggs irradiated in air. Upon interrupting diapause by cold treatment for three months when returned to 25°C. these embryos irradiated under N_2/CO_2 unlike those irradiated in air show development. Namely, they undergo blastokinesis, increase in size, resting nuclei appear normal, respiration is normal, mitosis resumes, the embryos grow, and the oxidative enzyme due to X radiation does not appear. These do not hatch however. Since any oxygen which is dissolved in the egg is theoretically used up in 1/2 hour the irradiation effect on these embryos under N_2/CO_2 is in complete metabolic standstill. Evidently in orthoptera no anaerobic glycolysis takes place, so that no form of energy release known to us is possible. Since the oxygen substrate moiety is interrupted we may regard metabolism stopped and under such conditions irradiation damage is at a minimum.

16. OXIDASE INCREASE IN *MELANOPLUS DIFFERENTIALIS* EGGS CAUSED BY X-IRRADIATION. Theodore N. Tahmisian and Dorothy M. Adamson, Argonne National Laboratory. (Lantern; $7\frac{1}{2}$ min.)

When diapause eggs of *Melanoplus differentialis* are X-irradiated (200 kv, 15 ma) with dosages ranging between 25,000 r and 200,000 r, the hydroquinone oxidase activity in the eggs is decreased immediately after the exposure. The enzyme activity increases with time so that the highest activity is obtained approximately 8 days after exposure. Oxidase activity after irradiation varies in the following sequence: 25,000 r > 50,000 r > 100,000 r > 200,000 r > control. The oxidase is inhibited by cyanide, carbon monoxide, and heat. Its activity is not affected by azide, phenylthiourea, nor diethyldithiocarbamate. The oxidase is specific for hydroquinone, and, in addition, it appears to have some effect on p-phenylene-diamine. It does not oxidize the —SH group in glutathione nor cysteine. Resorcinol or tyramine hydrochloride is also not oxidized by the enzyme. The nitroprusside test reveals that the native —SH groups are not oxidized as long as 124 hr after X-irradiation. However, by 18 days practically all —SH groups are absent. The increase in hydroquinone oxidase may be due either to the abolition of check mechanisms that normally repress oxidation or to an increase in the oxidase itself resulting from the breakdown of zymogens in the egg.

Embryology

Section B

17. HEAD CAVITIES OF THE HUMAN EMBRYO. Perry W. Gilbert, Cornell University. (Lantern; 15 min.)

The extrinsic ocular muscles of many of the lower vertebrates are foreshadowed in development by conspicuous spaces in the head region, known as head cavities. Three pairs of such epithelium-lined spaces, termed the premandibular, mandibular, and hyoid head cavities, have been described in elasmobranchs, some reptiles, and a few birds. In marsupials only premandibular head cavities are present, and from their walls the eyeball muscles innervated by the oculomotor nerve arise. With one exception, head cavities have never before been observed in a placental mammal and in that instance (a human embryo of 3.5 mm described by Zimmermann, '99) they were incorrectly interpreted.

Recently, in eight human embryos of 3-5.5 mm, belonging to horizons XII-XIV of the Carnegie Laboratory of Embryology collection, one to eight small cavities have been found on each side of the head in the premandibular mesoderm derived from the prechordal plate. The cavities, oval or spherical in shape, are usually situated immediately lateral and dorsal to the internal carotid arteries and are no larger in diameter than the height of the cuboidal or columnar epithelium lining them.

These cavities are believed to be homologous with the premandibular head cavities found in birds, reptiles, and elasmobranchs. As development proceeds they are replaced by premandibular condensations from which the extrinsic ocular muscles innervated by the oculomotor nerve arise.

18. DIFFERENTIATION OF VISCERA AND SURVIVAL OF TADPOLES WITH OPENED BODY CAVITIES. Norman E. Kemp, University of Michigan. (Lantern; 10 min.)

The ventral body wall posterior to the transverse septum was either completely or partially excised in tadpoles of *Rana pipiens* at various stages during the development of intestinal coiling. The entire ventral wall was removed either at the S-shaped stage of the digestive tract, after completion of 1 intestinal coil, or after completion of the larval pattern of intestinal coiling. One day after exposure of the viscera at the S-shaped stage, the animals were usually alive with blood still circulating through the gills; but the vitelline circulation had ceased. The mesoderm around the uncoiled gut had split and exposed endoderm was sloughing. The animals died, apparently from circulatory failure, during the second day, unless they were refrigerated. Vitelline circulation had ceased by 15 hours after operating at the stages of 1 intestinal coil or completed coiling. The tracts had uncoiled but were not split open, and they still showed peristaltic contractions. Peristalsis had ceased by 24 hours, and the animals likewise died during the second day unless refrigerated. If an anterior or posterior half of the coelomic body wall was excised at the S-shaped stage, those portions of the tract which remained covered by the remnant of body wall were protected from the deleterious effects of exposure. Such projected regions remained tubular,

whereas the exposed half burst open and underwent sloughing. The body wall apparently regulates or helps to maintain an osmotic pressure within the coelom which is favorable for normal circulation and normal differentiation of a tubular tract.

19. EFFECT OF GENE DOSAGE ON DEVELOPMENT OF PIGMENT PATTERN IN HETEROZYGOUS TRIPLOID AXOLOTLIS. H. Clark Dalton, Washington Square College, New York University. (Lantern; 15 min.)

Triploid axolotls heterozygous for the white-black pigment pattern alleles (*Ddd*) were produced by heat treatment of eggs from white females crossed with black males. Such triploid larvae showed fewer melanophores than *Dd* controls and failed to increase their melanophore number during a period when *Dd* larvae exhibited a considerable increase in number of melanophores. That this effect was not the result of triploidy itself was indicated by the fact that *DDD* larvae were not so distinguished from *Dd* controls. In the normal white pattern (*dd* animals) melanophores are fewer and less extensively distributed than in the black pattern (*DD* and *Dd* animals). Because *Ddd* larvae showed reduction in number of melanophores only, without restriction of their distribution, it appears possible that two activities of gene *d* are separate, at least to the extent that gene *D* may be completely dominant over *d* with respect to the mechanisms that determine distribution of melanophores, but not completely dominant over *d* (in *Ddd* animals) with respect to activities that determine melanophore number.

20. INDUCTION OF REGENERATION OF THE LIMB OF THE ADULT FROG BY AUGMENTATION OF THE NERVE SUPPLY. Marcus Singer, Harvard Medical School. (Introduced by G. B. Wislocki.) (Lantern; 15 min.)

The ability of the limb of the tadpole to regenerate declines and is lost during metamorphosis. However, regeneration may be evoked experimentally in adult frogs by supplying the amputation stump of the forelimb with a nerve supply in addition to the one it already possesses.

The entire sciatic nerve and its major branches were dissected free to the level of the foot and then deviated under the lateral body skin to the amputation surface of the forelimb of the same side. Regeneration of the limb resulted in most instances. Visible evidence of increase generally appeared 3 or 4 weeks after the operation. The growths varied in size. Some regenerates showed external signs of morphogenesis with a tendency to hand or finger formation. Initial histological studies revealed the development of cartilaginous skeletal elements with articulating surfaces; of muscle; of tendon and other connective tissue elements.

These experiments were stimulated by results of previous neurological studies of regeneration in the urodele amphibian (newt) in which it was demonstrated that a threshold quantity of nerve fibers is required in order that regeneration occur. The possibility arose that tissue changes occur in the course of metamorphosis of the frog which render the normal nerve supply inadequate to supply

the neural contribution essential for regeneration of the limb. In order to provoke a regenerative response it would be necessary to supplement the normal nerve supply with an additional quantity. This was accomplished in the manner described above.

21. MORPHOGENETIC ACTION OF CARCINOGENS IN LIMB REGENERATION. Alexander G. Karczmar and George G. Berg, Pharmacology Department, Georgetown University Medical Center, Washington 7, D. C. (Lantern; 15 min.)

Inhibitions by carcinogens of limb regeneration in larval salamanders was reported by us earlier. The current data deal with the effects of carcinogens (1) on early and late regeneration, (2) regression of denervated amputated limbs, and (3) regeneration of limbs treated prior to amputation. Larvae were either kept in water solutions of dibenzanthracene endo succinate (DBAS) and water suspensions of methylcholanthrene, or injected with oil solutions of methylcholanthrene and oil homogenates of dibenzanthracene. DBAS inhibited growth both in early and advanced regenerates (complete inhibition with concentrations from 20 mg % up). The differentiation proceeded even in limbs that regressed due to the treatment (40 mg %). Regression after denervation was not potentiated by carcinogens, whose action differs therefore from that of nitrogen mustard (Karczmar and Berg, Fed. Proc., vol. 9, 1950). Pretreatment of intact limbs inhibits regeneration for about two weeks, while inhibition of regenerating limbs is more persistent. Inhibition by injected methylcholanthrene in oil, similar to that of a 16 mg % DBAS solution, lasts over three months, indicating slow absorption from the also persisting oil droplet. Oil homogenates of dibenzanthracene (less oil soluble than methylcholanthrene), and aqueous suspensions of methylcholanthrene (40 mg %) were not active. The carcinogens seem thus to act almost exclusively on the proliferation of the regenerate in contrast to the more diversified action of radiomimetics.

22. TUMOR AGENTS AS MODIFIED DIFFERENTIATED COMPONENTS OF CELLS.¹ S. Meryl Rose and Florence C. Rose, University of Illinois. (Lantern; 15 min.)

There is evidence that tumor agents are modified components of differentiated cells. Ordinarily non-cellular extracts of Lucké renal carcinomas contain an agent so differentiated that it can grow only in renal epithelial cells, causing them to become neoplastic. However, renal carcinomas of frogs occasionally induce regenerating cartilage or cartilage of young *Triturus* to become neoplastic. The modified agent from *Triturus* chondrosarcoma can in turn induce cartilage to become neoplastic in frogs. Four times, once in a salamander and three times in a frog, a primary tumor induced by a modified agent was followed by a secondary tumor at the same joint in the opposite limb. This indicates that a growth promoting fraction of the original frog renal tumor agent had first become a part of regionally specific chondrocyte substance in a salamander and then when passed back to frogs had transferred to a substance differentiated

for a different region. The newest tumor agent could grow not only in cells on the side of the body where it was produced but could also transfer and grow in the identical spot on the other side of the body. In another case the agent from a renal tumor being cultured in an eyechamber transferred to iris cells. The combination renal and iris tumor when transplanted to the left eyechamber of another frog regressed, but shortly thereafter an iris tumor appeared in the right eye.

There are sufficient data in the literature to lead one to suspect that these results are interpretable as cases of transfer of a growth promoting fraction or locus from one differentiated nucleoprotein to another followed by the reproduction of the new combination.

¹ This work has been aided by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

23. THE EFFECT OF FLANK AND GILL TISSUES ON THE ORGANOGENESIS OF THE URODELE LIMB. Charles E. Wilde, Jr., School of Dentistry, University of Pennsylvania. (Lantern; 15 min.)

When limb buds from stages 30 to 40- (Harrison) are explanted to nutrient medium their morphogenetic expression is limited to the formation of a humerus. If peribrachial flank tissue is included with the limb bud much more complete organogenesis ensues. If gill primordia in semi-quantitative amounts are cultured with the limb bud, the gills develop exceedingly well but the limb is retarded, particularly in its distal differentiation. The effect of the gill tissue on the limb is roughly quantitative, and increases with amount of gill tissue present. More axial trunk structures of the limb level differentiate well but have no appreciable effect upon limb organogenesis in vitro.

The gill suppression of limb has been confirmed in vivo by transplanting limb and gill primordia so that the limb develops in a nest of gills. Various orientations have been used. Up to stage 33 the limb bud grows as a suppressed spike with little distal differentiation. At stages 34 and 35 the suppressor effect is labile. At stage 36 the limb develops to maturity unaffected by gill growth activity.

The loss of limb suppression effect by the gills is correlated with time of fixation of the D-V and M-L axes of the limb. The flank control of proximo-distal appearance of competences for formation of distal limb parts is associated by site with flank control of axes of symmetry of the developing limb.

The interaction of the various stimuli is interpreted as part integrating mechanisms active in controlling rates and sequences of normal embryogenesis.

24. AN ANALYSIS OF THE SPATIAL DISTRIBUTION, TRACT SPECIFICITY, AND ORIENTATION OF FEATHER GERMS IN THE HUMERAL TRACT OF THE CHICK WING. John W. Saunders, Jr.,¹ Marquette University. (Lantern; 15 min.)

To ascertain the time at which the characteristics of the humeral feather tract become fixed, rectangular isolates (ectoderm with underlying mesoderm) (ca., 0.34 by 0.50 mm, average) from the dorsal side of the 3-4 day wing bud (stages

4-10; Saunders, '48), consisting, in part, of prospective humeral tract, and in part, of the adjoining alar tract, were rotated 180° and replaced. Thus, after rotation, tissues normally forming the early emerging and structurally distinct humeral feathers developed distally on the limb where the later-emerging wing coverts appear.

Sixty-six cases, including embryos and adults, were examined. Of these, 29 showed feathers arranged in typical tracts, indicating complete regulation of the reoriented grafts. Atypical cases, all showing tracts deficient in feather germs, are classifiable in 3 groups: (1) those having no additional defect, indicating complete regulation of the distally-displaced tissues (from operations in all stages, 4-10); (2) those cases showing normally oriented humeral feathers (identified by structure and time of emergence) displaced distally on the upper arm, denoting regulation of the polarity of the reoriented isolate (from operations in stages 6-10); (3) cases similar to (2), but having the apices of the displaced humeral feathers directed anteriorly or laterally, indicating little or no regulation of the reoriented tissues (from operations in stages 8-10).

These results suggest that during the fourth day of development: (1) the delimitation of the humeral tract, (2) the characteristic structure and time of emergence of the humeral feather germs, and (3) the orientation of these feathers with respect to the body axis, are fixed in the sequence indicated.

¹ Work initiated at The Johns Hopkins University under the direction of Professor B. H. Willier.

25. THE EFFECTS OF RADIATION ON THE PREIMPLANTATION STAGES OF THE MOUSE EMBRYO. Liane Brauch Russell and W. L. Russell, Oak Ridge National Laboratory. (Lantern; 15 min.)

Irradiation during preimplantation (cleavage) stages causes high prenatal mortality but only a negligible incidence of abnormalities in animals surviving to term, the relative magnitude of these two effects being the reverse of that found following irradiation of postimplantation stages. To determine time of death, inbred females which had received 200 r on day $\frac{1}{2}$, $1\frac{1}{2}$, $2\frac{1}{2}$, $3\frac{1}{2}$, or $4\frac{1}{2}$ after mating were dissected on day $10\frac{1}{2}$ or $13\frac{1}{2}$ of gestation. A control was handled simultaneously with each irradiated female. Altogether 831 embryos and resorbing bodies were observed. In overall effect, the earliest stages are more sensitive: the average number of living embryos per treated female is approximately 20% of the controls in groups irradiated on days $\frac{1}{2}$, $1\frac{1}{2}$, or $2\frac{1}{2}$, but 31% and 57% following treatment on days $3\frac{1}{2}$ and $4\frac{1}{2}$ respectively. In groups irradiated on day $2\frac{1}{2}$ and $3\frac{1}{2}$, the major part (81% and 53% respectively) of the reduction in live embryo count is accounted for by death after implantation; whereas in groups irradiated on day $\frac{1}{2}$, $1\frac{1}{2}$, and $4\frac{1}{2}$, the majority of the deaths (71%, 71%, and 75% respectively) occurs before implantation. Postimplantation death probably occurs considerably before day $10\frac{1}{2}$. The main or sole expression of preimplantation death is on entire litters in groups other than those irradiated on day $\frac{1}{2}$ or $1\frac{1}{2}$ where 39% and 22% respectively of the preimplantation death is due to selective mortality within litters. Total-litter death may be due to radiation effect on the mother rather than direct lethal action on embryos.

26. THE EXPRESSION OF A DOMINANT GENE IN THE DEVELOPMENT OF THE PIGMENT PATTERN OF *RANA PIPENS*. Alice S. Baker, The Florida State University, Tallahassee, Florida. (Introduced by John A. Moore.) (Lantern; 15 min.)

The inheritance of the spotted pattern in the frog, *Rana pipiens*, has been analyzed (Moore, '42) and offers suitable material for correlating genetical, developmental and biochemical studies. Characteristics known to affect the formation of pigment pattern have been studied in adults of two genotypes, the wild type *R. pipiens* (homozygous recessive *bb*) and the non-spotted burnsi mutant (heterozygous *Bb*). The only characteristics studied which were found to differ between *pipiens* and *burnsi* skin are the quantity and distribution of melanin, the number and distribution of melanophores, and the amount of tyrosinase. The differences in melanin and melanophores exist only in the spots of *pipiens*, and the background regions are similar in these characteristics to the *burnsi* skin. The spots contain more melanin, fewer epidermal melanophores, and more intracutaneous melanophores, than either the background regions of *pipiens*, or any region of *burnsi* dorsal skin. As for tyrosinase, there is higher activity throughout all parts of *pipiens* skin than in any part of *burnsi* skin. No differences were found between any parts of *pipiens* and *burnsi* skin in dopa oxidase activity, the amount of tyrosinase inhibitor (discovered during these researches to be present in frog skin) or free substrates.

The expression of the *burnsi* gene in the adult frog is discussed in terms of a single primary gene effect, or of pleiotropic action.

27. REVERSAL OF PROXIMO-DISTAL POLARITY IN LIMBS OF ADULT NEWTS. J. N. Dent, University of Virginia. (Lantern; 12 min.)

It has been reported (Butler, 1949) that the proximo-distal polarity of limbs is reversible in the larvae of *Amblystoma opacum* and *A. punctatum*. Application of the technique of Butler, which involves a transplantation such that the original proximal end of the limb becomes the free end of the transplant, to adults of *Triturus viridescens* produced in some instances well-developed limbs with reversed proximo-distal polarity. Other transplants did not grow but persisted for 4-6 months without appreciable indication of regression. Still others, during a similar period of time were completely (or almost completely) resorbed. In a second series of transplantations a nerve from another limb was deviated to lie beneath the pocket into which the transplant was inserted. Such transplants produced limbs of reversed polarity in considerably shorter periods of time than transplants to which nerves had not been deviated.

Endocrinology

Section C

28. INFLUENCE OF ESTROGEN ON THE REPRODUCTIVE SYSTEM AND FERTILITY OF 10 DAY OLD MICE. J. H. Leatham and R. J. Merklin, Rutgers University. (Lantern; 15 min.)

One subcutaneous injection of estradiol dipropionate (Ciba) in dosages of 1, 2, 10, 50 and 250 μ g was administered to 10 day old mice. The two largest

doses retarded body weight increase in comparison with littermate controls at 10, 20, 30 and 40 days after injection. Ovarian weight was retarded by $2\text{ }\mu\text{g}$ or more and the $1\text{ }\mu\text{g}$ dose delayed the appearance of corpora lutea for 10 days. In the male, $1\text{ }\mu\text{g}$ retarded testis weight increase but recovery was attained in 40 days. Spermatogenesis was delayed by 10 days. The $10\text{ }\mu\text{g}$ dosage prevented the testis from reaching control weight after 40 days and spermatozoa were not present. The 40 day recovery period was the longest studied to date. Fertility of injected females was tested 18 to 25 weeks after the single injection. Normal litters were obtained from 14 mice injected with $1\text{ }\mu\text{g}$ and the 14 littermate controls. However, only 6 of 14 mice injected with $10\text{ }\mu\text{g}$ weaned litters, 2 gave birth and killed the young whereas 6 failed to become pregnant although in breeding for 70-80 days. All 15 littermate controls of this latter group weaned normal litters.

29. INFLUENCE OF RELAXIN ON MAMMARY DEVELOPMENT. Fern A. Garrett and R. V. Talmage, The Rice Institute. (Lantern; 10 min.)

These studies concern the nature of the possible influence of relaxin upon the development of the mammary gland, and were carried out utilizing both castrated immature female guinea pigs and rabbits. The results include only those animals treated 8 to 10 days with estrogen followed by a combined treatment of estrogen and relaxin for an additional 10 days.

In the guinea pig, in which estrogen is able to stimulate both duct and alveolar development in the mammary glands, a definite quantitative increase in the degree of development was achieved by the addition of relaxin to the estrogen treatment. Estrogen in the rabbit, however, normally stimulates only duct development. In young castrates of this species, the hormone ($5\text{ }\mu\text{g}$ estradiol/day) rarely produces changes during the first twenty days of treatment other than in the primary duct system which becomes enlarged far out of proportion to the rest of the gland. The addition of relaxin during this period appears to transfer the emphasis of growth from these primary ducts to the entire ramifying duct system, doing this without inducing any significant alveolar growth. Work now in progress indicates that this same shift of emphasis can be produced by estrogen alone if treatment is carried on for more than twenty-five days.

From these studies it is postulated that the action of relaxin in respect to mammary growth is in the role of a potentiator of estrogen rather than producing specific changes attributable to relaxin per se.

30. B-GLUCURONIDASE ACTIVITY AND TISSUE GROWTH. E. Knobil, Cornell University. (Introduced by H. B. Adelman.) (Lantern; 10 min.)

The growth of uterine tissue induced by the action of estrogens is accompanied by a rise in B-glucuronidase activity has been clearly demonstrated by previous investigations. The present work shows that tissue proliferation effected by other sex hormones is not necessarily paralleled by a similar rise in the concentration of this enzyme in the target organ studied.

The B-glucuronidase activity of prostates and seminal vesicles of intact and castrated male rats were compared. Although these organs in intact males were ten and four times larger respectively than those in castrates, no significant difference in their enzyme levels was detected.

The B-glucuronidase activity of the rabbit uterus on the sixth day of pseudopregnancy, when progestational proliferation was extensive, did not differ from that observed in the estrous uterus.

Deciduomata in the pseudopregnant rat had a higher enzyme activity than the remainder of the uterine horn devoid of these structures, but, despite the intense proliferative nature of the decidual reaction, this activity did not significantly exceed that found in the uteri of cyclic animals.

It is proposed that a specific relationship may exist between increases in B-glucuronidase levels and growth induced by estrogenic substances.

31. A STUDY OF THE UTERINE B-GLUCURONIDASE LEVELS IN THE RAT. S. L. Leonard and E. Knobil, Cornell University. (Lantern; 15 min.)

The enzyme B-glucuronidase, present in many tissues, was found to decrease in concentration in the mouse uterus after castration and to increase upon administering estrogens (Fishman, Levy and others). Following their techniques and using the bisynthetic substrate phenolphthalein glucuronide it was found that hormonal changes in the estrous cycle of the rat were not accompanied by alterations in the uterine B-glucuronidase levels. Spayed rats showed a decrease in uterine weight before the first significant decrease in the concentration of B-glucuronidase occurred 8-12 days after spaying. By 30 days the enzyme concentration reached a constant low level.

Using 20-day spayed rats, a single injection of .83 γ of estradiol benzoate produced a significant rise in the uterine enzyme by 72 hours. Larger doses, to 41.5 γ did not increase it further. Single injections of 4.15 γ of estrogen significantly increased the uterine weight by 12 hours but the enzyme concentration did not increase until 48 hours. Daily doses of 8.3 γ of estrogens for 20 days increased the enzyme level slightly but significantly above that in the intact female.

B-glucuronidase in the uterus of a 9-day pseudopregnant rat was significantly lower than that in the cyclic rat. However, artificially produced deciduomata had a higher enzyme concentration, about equal to that of the normal cyclic female. The time lag between the uterine weight changes and enzyme levels with alterations in circulating estrogens explains in part the failure to detect cyclic changes in the enzyme level during the estrous cycle.

32. EFFECT OF ESTROGEN ON GENITAL PHOSPHATASE ACTIVITIES IN THE MALE GUINEA PIG. Howard A. Bern, University of California, Berkeley. (Lantern; 8 min.)

Sexually mature male guinea pigs were castrated and allowed to remain for 3½ months with and without continuous estrogen treatment by subcutaneous implantation of estradiol pellets. Portions of the vas deferens, seminal vesicle

and prostate were homogenized, and alkaline and acid phosphatase activities were quantitatively determined by the method of Huggins and Talalay. Additional tissue segments were fixed in acetone and examined for the cytochemical localization of alkaline phosphatase by the method of Barger.

Castration results in a significant decrease in alkaline and acid phosphatase activity of the seminal vesicle. Estrogen treatment of castrates results in acid phosphatase activities lower than normal in all three organs tested and lower than castrate values in the vas and the seminal vesicle. Although seminal vesicle alkaline phosphatase values are similar in intact and estrogen-treated castrated animals, cytochemical studies reveal a shift in localization from the epithelium to the subepithelial stroma and fibromuscular wall, with the exception of certain metaplastic epithelial areas. In general, the fibromuscular hypertrophy in all organs due to estrogen is accompanied by increased alkaline phosphatase activity. The possible significance of these alterations will be discussed.

33. INFLUENCES OF ADRENAL CORTICAL SECRETION ON VITAMIN A METABOLISM IN THE RAT. Calvin T. Klopp, George Washington University School of Medicine. (Introduced by Dr. Warren Andrew.) (Lantern; 10 min.)

Administration of cortisone acetate, desoxycorticosterone and ACTH to humans was found to influence the serum vitamin A concentration. To determine the mechanisms of these responses, rats were studied. Rats were adrenalectomized and sacrificed in groups of 6 at 24 intervals, blood for vitamin A determinations being obtained by cardiac puncture at time of sacrifice. Control rats were subjected to a sham operation and pair fed with adrenalectomized mates. Vitamin A was found to practically disappear from the blood stream within 5 to 10 days following adrenalectomy, despite parenteral administration of the vitamin in amounts which maintained normal blood values in the control animals. Additional studies are now being conducted to determine the effect of adrenalectomy on concentration of vitamin A in liver tissue and the effect of administration of adrenal hormones on both blood and liver concentrations of vitamin A in the adrenalectomized and control rats.

34. REINVESTIGATION OF REPRODUCTIVE FUNCTIONS OF PITUITARY STALK-SECTIONED RATS. Roy O. Greep and Russell J. Barrnett, Harvard School of Dental Medicine, and Department of Anatomy, Harvard Medical School. (Lantern; 15 min.)

Reports conflict concerning whether the gonads are normal or atrophic following section of the pituitary stalk in experimental animals. This study describes the reproductive functions of 43 female and 23 male adult albino rats in which the completeness of stalk section was investigated by histological examination of serial sections through the stalk region, or dissection of India ink-injected heads.

Following stalk section no female rats had regular vaginal cycles. In over 50 days of observation, less than 20% showed at most 1 to 4 irregularly spaced periods of vaginal conification, whereas, the remainder showed either estrous smears for a few days postoperatively and none thereafter or complete absence

of cornification. Ovarian weights of stalk-sectioned rats were 52% of control values. Microscopic examination revealed a substantial degree of ovarian atrophy. Primordial and small growing follicles were always present but mature graafian follicles were rare. In most rats, persisting corpora lutea were found, in others, none. The ovaries displayed less sudanophilia than those of controls. Uterine weights averaged less than half the control values, and the vaginal epithelium assumed the histological appearance characteristic of diestrus or immaturity.

Male stalk-sectioned rats exhibited testicular atrophy, moderate to severe in degree, with retraction of the testes from the scrotum. The prostate glands and seminal vesicles were diminished in size. Experiments are in progress to assay the gonadotropic potency of the pituitaries of stalk-sectioned male rats.

35. EVIDENCE OF PANHYPOPITUITARISM IN PITUITARY STALK-SECTIONED RATS.

Russell J. Barnett and Roy O. Greep, Department of Anatomy, Harvard Medical School, and Harvard School of Dental Medicine. (Introduced by Helen W. Deane.) (Lantern; 15 min.)

Infundibular stalk section reportedly interrupts the pathway by which environmental influences modify pituitary activity. We could substantiate that the thyroid response to cold (as measured by cell height index) in 23 rats was not fully obtainable after stalk section. However, contrary to previous reports, the thyroids of 20 stalk-sectioned rats kept at room temperature were hypoactive. Furthermore, the thyroid response of 17 such animals to thiouracil feeding (0.5% of diet) was 8% of the normal response. The pituitary glands of these rats showed no hypertrophy nor increase in basophils.

The condition of the adrenal glands and gonads of the stalk-sectioned rats indicated a state of panhypopituitarism. At room temperature, the average adrenal weight of 22 stalk-sectioned rats was 48.8% of controls, whereas, after exposure to cold the average adrenal weight of 23 operated rats was 41.1% of controls. Thirty-four per cent of stalk-sectioned rats succumbed to cold stress. Besides decreased width of the fasciculata and reticularis and diminished sudanophilia in comparison to controls the adrenals of female stalk-sectioned rats revealed a prominent transition zone.

Mallory-Azan stained sections of pituitaries of stalk-sectioned rats revealed central necrosis and scarring, decreased numbers and granulation of acidophils and basophils and an increase in small chromophobes of uniform size.

We interpret our experimental findings as resulting from devascularization and ischemic necrosis of the adenohypophysis. Heretofore stalk section was believed to abolish responses to cold; the present experiments indicate that various pituitary functions are blocked regardless of environmental temperature.

36. LOCALIZED METAMORPHIC CHANGES IN THE SKIN OF RANA PIPIENS LARVAE BY SUBCUTANEOUS IMPLANTS OF MOUSE AND FROG THYROID GLANDS CONTAINING RADIOACTIVE IODINE. Jane Couffer Kaltenbach, The State University of Iowa. (Introduced by Titus C. Evans.) (Lantern; 15 min.)

Local metamorphic responses, ascribed to the direct action of thyroid hormone, have been demonstrated in skin and other regions of amphibian larvae by im-

plantation of thyroid glands and thyroxin-containing pellets (Hartwig, 1940; Kollros, 1942; Lüke, 1944; Kaltenbach, 1949). It is of interest to determine whether thyroid hormone in such cases is maintained in higher concentrations in regions of localized response than in the animal as a whole. I^{131} was injected subcutaneously into mice and frogs and was quickly incorporated into their thyroid glands. Two days later fragments of the glands were implanted beneath the dorsolateral skin of young *Rana pipiens* larvae. General metamorphosis was accelerated, and metamorphic changes in skin overlying the implants were especially accelerated as evidenced by localized thickening, stratification, cornification, molting, and glandular development. Radioautographs showed only slight local effects when I^{131} diffusion was rapid but marked effects when high concentrations were retained in the implants. At one day phagocytosis of the mouse implants was evident; I^{131} appeared in body fluids and was highly concentrated in the tadpoles' thyroid glands. Only slight changes occurred in the overlying skin. However, at one week some I^{131} remained in many mouse implants, and local skin changes were more advanced. In contrast, frog implants often became established. At two weeks I^{131} was highly concentrated in the follicles but not in the overlying skin, in which marked changes developed. The experiments demonstrate precocious induction of local metamorphic skin changes near subcutaneous thyroid implants containing relatively high concentrations of radioactive iodine.

37. THE ANTAGONISTIC EFFECT BETWEEN THYROXIN AND VITAMIN A ON ANURAN METAMORPHOSIS. Jerome J. Klenner and Joseph F. Gennaro, Jr., University of Pittsburgh. (Lantern; 10 min.)

An antagonistic effect between thyroxin and vitamin A has been reported. Some have tried to adapt this vitamin to clinical use in treatment of the hyperthyroid condition. (Euler, H. and E. Klussman, *Zeitschr. f. Physiol. Chem.*, 1932, 213, 27 and Simpkins, S., *J. Clin. Endocrinology*, 1947, 7, 574.) In view of the many contradictory views of other observers, experiments are presented here which suggest that the effects of simultaneous administration of massive doses of vitamin A on the thyroxin-induced metamorphosis of anuran tadpoles (*R. clamitans*), are negligible.

Changes in total body length, hind limb-bud length, and weight were recorded for one hundred and sixty-six tadpoles of early limb-bud development. Thyroxin was administered in a concentration of one part hormone to five million parts of natural spring water, and vitamin A (β -carotene in oil) was injected intraperitoneally (18,750-70,000 I.U. per larva).

The results of this treatment indicate that: (1) thyroxin, in the concentration employed here, significantly accelerated the development of the tadpoles immersed in it beyond that of the controls in pure spring water; (2) vitamin A administered without the thyroid hormone did not affect the rate of larval change; (3) there is no significant difference between the development recorded for the individuals under thyroid treatment alone and those treated with both the hormone and the vitamin A.

Considering the toxicity of these substances the proposed antagonism would warrant the expectation that, used together, some of the ill effects of both would

be lessened. But, in no instance was the death rate of the thyroxin-vitamin A group significantly lower than that of the groups subjected to the vitamin or hormone alone.

38. SOME EFFECTS OF PHENYLALANINE AND TYROSINE DEFICIENT DIETS ON THE RAT. Earl B. Scott, Charles Schwartz, and Ralph Ferguson, University of South Dakota, School of Medicine.¹ (Lantern; 15 min.)

Groups of 30 day-old male rats were placed on normal, phenylalanine deficient, tyrosine deficient and phenylalanine-tyrosine deficient diets. The absence of tyrosine had no effect. The lack of phenylalanine or phenylalanine and tyrosine together produced similar changes and the results are described together for simplification.

Pronounced atrophy of the testes, epididymides, prostates and seminal vesicles developed in all animals. The seminiferous tubules were extensively damaged; spermatozoa were absent. The testicular interstitial cells were present in numbers but altered in morphology, resembling fibroblasts. The atrophic state of the accessory reproductive glands presented evidence that the interstitial cells were functionally inactive.

Eight of ten rats showed distinct alteration of the glomerulosa of the adrenals. The cells of this area were closely packed and poor in cytoplasm. Other areas of the adrenal glands were unchanged.

The thyroids were smaller than normal. The epithelium was not markedly lower than normal, but the follicles were quite variable in size, with and without colloid.

The thymus glands of the deficient rats were extremely atrophic. No attempt was made to study them histologically.

The acidophils of the anterior hypophysis were distinctive by their scarcity of cytoplasm and poor staining reaction. The basophils were fewer and fainter, but unaltered in size. The pituitaries of those deficient rats whose adrenals were unaltered appeared normal.

Bone growth was greatly reduced as evidenced by examination of the epiphyseal cartilage of the tibia.

All other structures appeared histologically normal.

¹ This work was supported in part by a grant from the United States Public Health Service.

Cytology and Protozoology

Section D

39. FEULGEN STUDIES OF ISOLATED NUCLEI FOLLOWING TREATMENT WITH HEPARIN. Henry S. Roberts, Jr. and Norman G. Anderson, Duke University. (Introduced by George T. Hargitt.) (Lantern; 15 min.)

Rat liver nuclei isolated by the method of Anderson and Wilbur were stained by the Feulgen reaction in an effort to demonstrate cytologically the presence of desoxyribose nucleic acid (DNA) in the gel produced, and to determine the structural changes resulting from heparin treatment. In heparin treated nuclear prep-

arations fixed with acetic alcohol or Allens B15 no clear Feulgen positive reaction was obtained in the extra-nuclear gel, although Anderson and Wilbur have shown by chemical tests that this gel consists of DNA or a DNA-protein complex. Formalin failed to coagulate or precipitate the DNA gel and so could not be used satisfactorily for fixation. When the Feulgen reaction was used without prior fixation a strongly positive color reaction resulted in the extra-nuclear gel. An excellent intra-nuclear stain however was obtained with and without prior fixation. Liver nuclei showed marked internal vacuolization and paler staining after heparin treatment. White blood cells with visually intact cell membranes present in the preparation showed no comparable effect of heparin. Evidence was obtained which suggests that heparin combines with histone of the nuclei, releasing DNA and resulting in the structural damage observed. The possibility exists that the Feulgen test, after ordinary fixation, depends to some extent on the manner in which the DNA is bound.

40. A DIFFERENTIAL STAIN FOR RIBONUCLEIC AND DESOXYRIBONUCLEIC ACIDS. Martin H. Flax¹ and Marion H. Himes, Columbia University. (Introduced by Arthur W. Pollister.) (Lantern; 15 min.)

A new dye compound is formed when potassium acid phthalate is added to the basic stain, Azure A. This compound, called for convenience Azure-phthalate, is, like Azure A, a metachromatic dye; that is, in solution its absorption peak is shifted towards shorter wavelength when the dye concentration is increased or when the dye is combined with metachromatic substances. The absorption spectrum of Azure-phthalate is similar to that of Azure A, but histological staining properties of the two (at pH 4) are quite different. While Azure-phthalate retains the specificity of Azure A for nucleic acids and other strongly acidic substances, such as some esters of polysaccharides, it differs from Azure A in staining differentially the two nucleic acids—ribonucleic acid (RNA) staining red and desoxyribonucleic acid (DNA) blue. This specificity is demonstrated by the fact that the blue staining of nucleic acid is removable by desoxyribonuclease and the red staining by ribonuclease. The parallel between this red color and that resulting from combination with half-esters of polysaccharides suggests that RNA, like the more familiar metachromatic substrates, may provide adjacent sites for dye attachment which are so oriented as to allow dye interaction. Preliminary experiments are consistent with the view that the basis of color differentiation lies in the difference in molecular structure of DNA and RNA, the latter having many more secondary phosphoryl dissociations, which with adjacent primary dissociations may enable it to serve as a metachromatic substrate. At pH 3, RNA is stained blue; the color shifting toward red as the dye solution is made more alkaline. These changes may be associated with increasing combination between dye and secondary phosphoryl groups as the pH increases. Azure-phthalate appears to be a promising tool for localizing nucleic acids and studying their structure.

¹ Predoctoral fellow, National Cancer Institute, U. S. Public Health Service.

41. A CYTOLOGICAL STUDY OF TUMOROUS GROWTHS IN A STRAIN OF *DROSOPHILA MELANOGASTER*. Ernest W. Hartung, Rhode Island State College. (Lantern, 12 min.)

A cytological study was made of the tumorous masses found in larvae of the *Drosophila melanogaster* stock, bw mt⁴ vg. Larvae were fixed in Henning's fluid, sectioned and stained, and the tumors examined in situ. The masses are cellular, being comprised of small cells having large nuclei and prominent nucleoli. Little or no evidence of mitotic activity is apparent and the growth in all cases appear as discreet nodular clumps rather than as invasive proliferations. There appears to be little restriction as to the locale in which the masses appear, hence they are not especially associated with any one organ. The melanin sheath which characterizes the growths is extra-cellular and usually appears late in their developmental history. As far as can be ascertained the criteria for neoplasia are not met in these tumors and their designation as benign cellular masses would appear to be correct.

42. A CYTOCHEMICAL STUDY OF OOGENESIS AND CLEAVAGE. Max Alfert, University of California. (Introduced by Curt Stern.) (Lantern; 15 min.)

Photometric measurements of desoxyribonucleic acid (DNA) and protein were made on developing oocyte nuclei, pronuclei and early cleavage nuclei of the mouse, making use of the Feulgen reaction for DNA and the Millon reaction for tyrosine and tryptophane. Changes in nuclear volume (increase during oogenesis and decrease in the course of cleavage) could be correlated with parallel changes in protein content. The amounts of DNA per nucleus however do not change with nuclear volume: Primary oocyte nuclei contain a constant amount of DNA which is progressively diluted during growth of the oocyte. Pronuclei carry one-fourth of the amount of DNA present before meiosis in oocytes, and this amount is doubled prior to the first cleavage division. Nuclei of succeeding cleavage stages all carry twice the amount of DNA present in early pronuclei and this is always doubled prior to onset of nuclear division. It is concluded that a constant amount of DNA is associated with a haploid set of chromosomes and that no changes in amount other than those correlated with duplication or reduction of the chromosome complement occur.

In the course of oogenesis and cleavage striking changes in Feulgen stainability of nuclei arise. These are due to a dilution and concentration effect, respectively, of a constant amount of stainable substance dispersed in nuclei of varying volumes. Such changes in stainability were previously misinterpreted to indicate variable amounts of DNA per nucleus at these stages.

43. FURTHER DATA ON "TRIPLE REPEATS" IN *SCIARA*. C. W. Metz and J. Paul Reynolds, University of Pennsylvania. (Lantern; 10 min.)

As reported in earlier papers, a "triple repeat" condition normally present in the X-chromosome of *Sciara ocellaris* and *S. reynoldsi* (two species which will hybridize) appears to possess special evolutionary interest because it suggests

that qualitative change has occurred in two of the three duplicated segments (r^1 , r^2 , r^3) subsequent to their origin in an ancestral species. This interpretation is based on the fact that the three regions, which are far apart in the chromosome, exhibit selective synaptic affinities as shown by study of the salivary gland chromosomes. Sections r^2 and r^3 regularly undergo synapsis with r^1 , but not with one another, suggesting that they have undergone qualitative divergence.

This interpretation, of course, rests on the validity of the evidence that the repeat regions are structurally alike and that synaptic attachments between r^1 and r^2 are like those between r^1 and r^3 . Considering the inherent difficulty of studying such details in repeats, on account of distortions due to "squashing" the material, it was felt desirable to extend the detailed morphological study. This has now been done on a scale which appears adequate and the results appear to confirm fully the earlier observations. The data will be presented and discussed briefly.

44. THE ACTION OF ALDEHYDE-COUPLING REAGENTS ON THE FEULGEN REACTION. I. GELATIN-DESOXYRIBONUCLEATE PREPARATIONS.¹ M. A. Lessler² and M. J. Kopac, Department of Biology, Washington Square College of Arts and Science, New York University. (Lantern; 15 min.)

Drops of 20% gelatin sol containing known amounts of Na desoxyribonucleate (DNA) were placed on albumin-coated microscope slides and allowed to gel. The slides were fixed in 6% formalin, washed, and subjected to a modified Feulgen procedure. The fixed preparations were exposed to aldehyde-coupling reagents (Na bisulphite, semicarbazide HCl, phenylhydrazine, hydroxylamine HCl, or trimethylaminoacetohydrazide) either before or after 10 minutes' hydrolysis in 1 N HCl at 550°C.

The Feulgen reaction was strikingly inhibited when these aldehyde-coupling reagents were applied after the DNA was hydrolyzed. Application of these reagents before hydrolysis of DNA produced negligible inhibition. Almost complete inhibition of the Feulgen reaction was produced with DNA concentrations up to 1 mg/cc. Inhibition was evident with DNA concentrations up to 5 mg/cc.

These results indicate that inhibition by aldehyde-coupling reagents occurs only when the DNA is hydrolyzed. It is reasonable to conclude that the Feulgen reaction involves a coupling between the Feulgen (Schiff) reagent and the aldehyde groups unmasked in the DNA by acid hydrolysis. If these groups are masked by aldehyde-couplers, then the Feulgen reaction is inhibited. In the absence of aldehyde-couplers, the Feulgen reaction occurs *in situ* providing that the DNA is bound to a protein.

¹ These investigations were supported by Grant C-843, National Cancer Institute, U. S. Public Health Service.

² Post-doctorate Fellow, National Cancer Institute, U. S. Public Health Service.

45. AN ELECTRON MICROSCOPE STUDY OF MITOSIS IN THE ONION ROOT TIP. Albert W. Sedar and Donald F. Wilson, The State University of Iowa. (Introduced by H. W. Beams.) (Lantern; 10 min.)

The electron microscope was used to study normal and colchicized onion root tips, placing emphasis on the spindle fibers. Material was usually fixed in Ran-

dolph's CRAF fixative to give good contrast between spindle fibers and clear background cytoplasm. Fleming's fluid with a minimum of acetic acid was also tried yielding more cytoplasmic detail and denser photographs, but without significantly altering the character of the spindle fibers. Sections were cut at approximately 0.5 micron from paraffin-embedded material using a Spencer 820 microtome with a thin section adapter.

Electron micrographs were obtained which confirm much of the cytology of the normal cell as disclosed with the light microscope. Spindle fibers form in the polar regions and extend to the nuclear membrane prior to its breakdown. No interzonal fibers were observed, but chromosomal fibers appear as compound structures. The cell plate seems to be formed by fusion of equatorial thickenings in the spindle fibers starting in the center and extending to the periphery.

The effect of threshold exposures to colchicine is a separation of the spindle fiber components, while prolonged exposure or exposure to higher concentrations results in fragmentation and partial dissolution of the fiber material.

46. ELECTRON MICROSCOPY OF THIN-SECTIONED SPIROSTOMUM. Harold E. Finley, Department of Zoology, Howard University, Washington, D. C. (Lantern; 10 min.)

The National Bureau of Standards microsectioning method was used in a study of the ciliated protozoan *Spirostomum ambiguum*. Fine details of many parts of thin-sectioned organisms were revealed by the electron microscope. Among the fine details of morphology disclosed, the following were prominent: Compact bundles of fibrils in locomotory cilia; basic architectural units of the ectoplasmic matrix (fibrils); basal bodies of membranelles and interciliary fibrils; particles in the macronucleus; and ingested organisms in food vacuoles. These fine structures will be illustrated and discussed.

47. SEXUAL DIFFERENTIATION IN RHABDOSTYLA VERNALIS.¹ Harold E. Finley and Philip A. Nicholas, Department of Zoology, Howard University, Washington, D. C. (Lantern; 10 min.)

In the peritrichous ciliates occurs a sexual type of reproduction which involves the union of a micro- and macroconjugant, the two organisms becoming one entity and offspring being produced therefrom. The authors' interest in such reproduction suggested the investigation of sexual differentiation in *Rhabdostyla vernalis*. Results obtained thereby indicate that sexual differentiation in *Rhabdostyla* is comparable but not identical to the phenomenon as it occurs in *Vorticella microstoma*.

Rhabdostyla's micro- and macroconjugant are derived from a "neuter individual" through an equal division of the micronucleus accompanied by an unequal division of the macronucleus and cytosome. The two products of this fission have different immediate fates although both are potentially destined for conjugation. The smaller product of the pre-conjugation fission is called a promicroconjugant; it does not seem to be completely differentiated at the same time as its sister macroconjugant. The promicroconjugant undergoes two consecutive fissions

while still attached to the macroconjugant. Thus each "neuter" *Rhabdostyla* usually produces one macroconjugant and four microconjugants. These preliminary results will be illustrated and discussed.

¹ This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

Cellular Physiology

Section A

48. FUNCTIONAL STUDIES ON MOLLUSCAN EPITHELIUM. E. Ronkin and R. R. Ronkin, University of Delaware. (Introduced by Secretary.) (Lantern; 15 min.)

P³²-labelled inorganic phosphate will pass from sea water into the ctenidial ciliated epithelium of *Mytilus*. At phosphate concentrations close to saturation an unexpectedly high rate of passage has been reported. It has been suggested that the epithelial cells may engulf particulate calcium phosphate present in the ambient solution. This raises the possibility that highly differentiated units such as ciliated cells may retain phagocytic capacity associated with cells having less structural differentiation. It should be possible to expose such tissues to solutions containing "tagged," insoluble particles, and later localize any intracellular particulate material that may have penetrated. Observations on molluscan epithelia from several sources are described, and these are discussed in relation to phagocytic activity and phosphate penetration.

49. EXPERIMENTS ON THE PHYSIOLOGY OF THE RUMEN. R. E. Hungate, Washington State College, and R. W. Dougherty, Cornell University. (Lantern; 15 min.)

A pH of 4.0 to 4.5 is found in the rumen of sheep dead from overeating grain. Acetic or lactic acid introduced into the rumen in amounts sufficient to give a pH of 3.2 causes an inhibition of the normal rumen motility that persists until the pH rises as the acid is absorbed. This is complete in about 8 hours. During absorption there is little change in the CO₂ combining power of the blood. Animals with an acidified rumen show inappetence and symptoms of discomfort, both of which disappear in 24 hours. The sheep recover completely. Most of the rumen protozoa are killed by the acid but not all and after a week the fauna is normal.

50. THE EFFECT OF CLOSTRIDIUM PERFRINGENS α -TOXIN ON THE OSMOTIC BEHAVIOR OF CHICKEN ERYTHROCYTES.¹ F. R. Hunter and Jane A. Bullock, Florida State University. (Lantern; 8 min.)

The effect of a partially purified preparation of α -toxin of *Cl. perfringens* (100–200 H.U./ml) on the osmotic behavior of chicken erythrocytes was investigated using a photoelectric technique. The following measurements were made: (1) rate of swelling of cells placed in 0.3 M glycerol in Ringer Locke solution; (2) rate of shrinking in 1.5 $\times$ Ringer Locke solution of cells previously equilibrated in 0.3 M glycerol in Ringer Locke solution; (3) per cent hemolysis (measured spectrophotometrically) of cells placed in solutions of NaCl of varying tonicities

(fragility measurements); and (4) cell volumes as determined using an air turbine. A comparison was made between erythrocytes exposed to a 1:500 dilution of the toxin and cells in the presence of Ringer Locke solution. In the presence of the toxin the cells swelled and were more fragile. The rate of movement of glycerol across the membrane, however, was not appreciably changed by the action of the toxin.

¹ This work was supported in part by grants from the Division of Research Grants and Fellowships, U. S. Public Health Service.

51. EFFECT OF VARIOUS INHIBITORS ON THE RESPIRATION OF *PARAMECIUM CAUDATUM*.¹ Elaine Nelson and Keatha K. Krueger, University of South Dakota, Vermillion. (Introduced by W. L. Hard.) (Lantern; 10 min.)

Studies on the respiration of whole organisms of *Paramecium caudatum*, variety 2, Type IV, using the Warburg respirometer, have been made. $Q_{O_2}(N)$ values for this organism paralleled closely those for other tissues. Both azide (0.01 M) and cyanide (0.001 M) inhibit respiration approximately 50%. Thiourea, which has a killing effect on *P. caudatum* at 0.1–0.5 M, at 0.0001 M has a very marked inhibitory effect on respiration without killing the organisms; this inhibitory effect could be almost completely removed by the addition of 0.1 or 0.01 M urea, and less strikingly removed by 0.01 M uracil. *P. caudatum* could become "acclimated" to thiourea (up to 0.02 M) and such cultures showed less inhibition of respiration by thiourea. Thiouracil (0.001 M) also inhibited the respiration of *P. caudatum*; this inhibition could be nullified to a great extent by 0.01 M uracil. The removal of the inhibitory effect of thiourea and thiouracil by urea and uracil suggests that it might be the same as that postulated for the metabolite-inhibitor relationships studied with other microorganisms, i.e., interference with the utilization of a specific substrate in an enzyme system. However, relatively large amounts of "metabolite" were needed to overcome the inhibition.

¹ This work has been supported in part by a grant from the U. S. Public Health Service.

52. THE NUCLEIC ACIDS OF THE LARVAL SALIVARY GLAND OF *DROSOPHILA ROBUSTA* AND THE POSSIBLE ROLE OF THE NUCLEOLUS. S. W. Leshner, University of Kansas. (Introduced by Hampton L. Carson.) (Lantern; 10 min.)

The nucleic acids of the salivary gland of *D. robusta* during the course of larval development were determined as to localization and as to type, i.e., DNA or RNA. The determination were made by the use of basic staining with toluidine blue, Brachet's ribonuclease method and the Feulgen reaction.

Measurements were made and volumes calculated of the nucleic and nucleoli in all stages of development. Cytological evidence indicates that the gland is functional as a secretory organ only during the first 170 hours of larval life. It is during this period that the cytoplasmic basophilia (RNA) is most intense and the nucleolus is increasing rapidly in size. When secretion ceases there is a simultaneous decrease in the other two, nucleolar size and RNA. These findings suggest that a close relationship exists between the nucleolus and the RNA system and that the RNA system is directly concerned with secretion.

Nuclear size, on the other hand, continues to increase beyond the 170 hour level of larval development. Furthermore, since the salivary gland chromosomes are definitely becoming more polytene, it would seem that a relationship exists between chromosome reduplication and nuclear size. In addition the evidence furnished by the nuclei-nucleoli measurements indicates that the behavior of the nucleolus does not necessarily parallel that of the nucleus.

53. ANTI-MITOTIC SUBSTANCES IN LIVING TISSUES. L. V. Heilbrunn and John W. Kelly, University of Pennsylvania. (Lantern; 15 min.)

As noted previously, it is thought that all cells contain some substances which tend to induce protoplasmic clotting and others which tend to prevent such clotting. The latter substances include heparins or heparin-like compounds. A survey of various types of cells indicates the widespread occurrence of substances which stain metachromatically with toluidine blue. Some of these substances are undoubtedly heparins, or at least they are heparin-like, and presumably they can act to prevent clotting. In view of the fact that the mitotic gelation is a clotting phenomenon, it should be possible to extract from the various types of living tissues substances which will inhibit the mitotic gelation and prevent the division of the cell. Such substances can indeed be extracted. By using acidified sea water, known to extract heparin-containing compounds from sea-urchin eggs, it was possible to extract from the worm *Chaetopterus*, from the clam *Macra*, and from various other living materials, substances which prevent mitotic gelation and cell division. The most potent extracts were obtained from the ovaries of the starfish *Asterias*. These extracts not only prevent mitotic gelation (and cell division) in the eggs of *Chaetopterus* and the sea-urchin *Arbacia*, they also cause a pronounced liquefaction of the protoplasmic cortex. Further studies will be made of the effect of starfish extract on other types of dividing cells.

54. RETARDATION THROUGHOUT THE MITOTIC CYCLE OF DIVIDING SEA-URCHIN EGGS BY THE "PROPHASE POISONS," IN URETHANS AND NITROGEN MUSTARDS. Ivor Cornman, George Washington University, Warwick Memorial Clinic. (Lantern; 12 min.)

The "prophase block" engendered by radiomimetic poisons when used on intact plants and animals is open to interpretations other than a specific interruption of mitosis at the prophase or preprophase. Sea-urchin eggs (*Arbacia*, *Lytechinus*, and *Echinarachnius*) exposed for several hours to concentrations of urethans or nitrogen mustards which markedly retard division nevertheless divide completely and repeatedly. Retardation is proportional to the duration of exposure, regardless of the mitotic phases traversed. Exposure to HN2 40 min. before the first cleavage or 40 min. before the second cleavage results in equal delay of the respective cleavage, even though the first entails exposure only of prophase, metaphase and anaphase whereas the second encompasses first and second prophases along with the intervening metaphase, anaphase and telophase. The picture is one of overall slowing, not inhibition of any one phase. A dose of HN2 added an hour before division at a concentration high enough to prevent

cleavage in 90% of the eggs holds only half the eggs at prophase. In the other half the nuclei dissolve. Some of these eggs divide but do not form blastomere nuclei, while some go through a second division and form four nucleated blastomeres. Thus HN2 treatment permits some eggs to consummate two prophases while other eggs are blocked at intervening phases. At lower doses which permit several cleavages, the blastomeres finally come to rest with the nucleus intact or represented by numerous karyomeres. Such a condition seems better designated not as a prophase block, but as an expression of general impotency, as attested by the eventual disintegration of the cell.

55. THE SITE OF ACTION OF ETHYL CARBAMATE AND SODIUM AZIDE IN THE LIVING EMBRYONIC CELL. Joseph Hall Bodine and Kiao-Hung Lu, State University of Iowa, Department of Zoology. (Lantern; 15 min.)

By means of fractional centrifugation techniques, the effects of ethyl carbamate and sodium azide on the oxygen uptake of the intracellular constituents can be compared with those for the intact grasshopper embryo. Ethyl carbamate and sodium azide produce a marked inhibitory effect upon the oxygen uptake of the intact embryo, its homogenate, the isolated intact nuclei, mitochondria and microsomes. Methylene blue antagonizes the inhibitory action of ethyl carbamate and sodium azide. 2-4 Dinitrophenol on the other hand produces a stimulating action only on the intact embryo and no indications of an antagonistic action are evident. Methylene blue exerts its stimulating action at the nuclear membrane while 2-4 dinitrophenol requires the cell to be intact to produce its stimulating effects.

56. THE RESPIRATION OF ISOLATED BLASTOMERES AND POLAR LOBES OF THE EGGS OF *MYTILUS EDULIS*. William E. Berg and Phyllis B. Kutsky, University of California (Berkeley). (Introduced by R. M. Eakin.) (Lantern; 15 min.)

The oxygen consumption of isolated blastomeres and isolated polar lobes of the eggs of *Mytilus edulis* has been measured by means of the Cartesian diver microrespirometer. The respiration of isolated AB embryos is 3.2×10^{-5} mm³/hr./embryo as compared to 5.6×10^{-5} mm³ O₂/hr./embryo for isolated CD embryos. If these rates are corrected for the initial amount of cytoplasm of the isolated blastomeres, the oxygen consumption of CD embryos is found to be significantly lower (11%) than AB embryos.

This lower respiratory rate of CD embryos has been found to be due to the presence of the polar lobe cytoplasm. The theoretical oxygen consumption of isolated polar lobes, based on the oxygen consumption of whole eggs during first cleavage, is 8.6×10^{-6} mm³ O₂/hr./polar lobe. Polar lobes isolated at first cleavage of the egg have an average oxygen consumption of 6.3×10^{-6} mm³ O₂/hr./polar lobe representing a 27% lower value than expected. This difference is significant and is sufficient to account for the lower respiration of CD embryos as compared to AB embryos.

The oxygen consumption of the polar lobe cytoplasm remains constant after isolation from the whole egg whereas the AB and CD embryos, as well as normal embryos, exhibit a gradual rise during subsequent development. Differences also exist in the shape of the respiration curves of the isolated AB and CD embryos during their first six hours of development.

57. EFFECT OF RADIATION FROM INTRAPERITONEALLY INJECTED RADIUM CHLORIDE UPON MEGAKARYOCYTE AND BLOOD PLATELET PRODUCTION IN ALBINO RATS. Paul G. Roofe, Hal Bingham, Ralph Comer and Martha Madison, University of Kansas. (Lantern; 5 min.)

Twenty-five rats were divided into groups of five, each group receiving respectively 10, 20, 30, 40, 50 μ g of radium chloride intraperitoneally. One rat from each group was sacrificed every third day starting three days post-injection.

Using a camera lucida, quantitative megakaryocyte counts were made from slides prepared from intact bone marrow of the femur. The platelet counts were made using a hemocytometer and a 2% sodium citrate diluting fluid.

Megakaryocytes decrease progressively with time through the ninth day after injection of radium chloride. From this period to the fourteenth day, a trend toward regeneration was noted. The lowest number of megakaryocytes was observed in animals receiving 20 μ g independent of time of sacrifice. Regeneration appeared earlier in the higher dosages.

Following an initial increase of platelets on the third day, there was a progressive decrease up to and including the fourteenth day. Regardless of time, higher dosages also produced a decrease from the normal.

The initial destruction of the megakaryocytes is reflected on the third day by an increase in platelets.

As the megakaryocytes regenerate it appears that platelet production does not correspond quantitatively, presumably because young megakaryocytes do not fragment.

General Demonstrations

Section B

58. LARVAL LAMPREYS FROM THE CHAMPLAIN AREA. Francis H. Wilson, Champlain College.

Lamprey larvae are plentiful and are excellent for presenting to students the cyclostomes as living animals.

59. COMPARISON OF TRANSPLANTABILITY OF SKIN, OVARY AND TUMOR IN MICE. Meredith N. Runner and Joy Palm, Rosecoe B. Jackson Memorial Laboratory.

Genetically tagged skin and ovary and two sarcomas have been transplanted within a single strain of mouse segregating for two coat colors. Skin was transplanted reciprocally and ovaries were transplanted in one direction between each

pair of females. Homotopic ovarian transplants enabled detection of successful grafts on the basis of appropriately colored offspring. Skin grafts were considered successful if the color and direction of newly formed hair conformed to that of the transplant.

Skin grew and persisted in 61 out of 73 transplants. Ovarian grafts were diagnosed as positive in 15 out of 22 attempts; more than 100 offspring have been reared from grafts. Some of the animals carrying skin grafts failed to reproduce young from ovarian transplants. Conversely animals with positive ovarian grafts have failed to support skin transplants. Sacromas on the contrary grow progressively and kill 100% of the recipients. Apparently factors which determine transplantability act independently in each of the three types of transplants.

Frequency of successful skin grafts was studied on the basis of closeness of pedigree and color of host. Grafts succeeded with similar frequencies in litter mate sibs as they did in animals separated for 8 or more generations and in hosts of either color. No obvious incompatibility factors were segregating either with the genes for coat color or with the different sublines. Study of succeeding generations should demonstrate whether the failure of ovary and skin to grow in all transplants is associated with Mendelian factors.

60. THE GOLGI APPARATUS AS REVEALED IN DESILVERED DA FANO PREPARATIONS. F. B. Adamstone, University of Illinois.

Osmic acid and Da Fano silver preparations of fresh frozen sections of nerve cells indicate that the Golgi apparatus is a cytoplasmic reticulum associated with mitochondria. Standard Da Fano preparations of fixed tissue produce a highly variable fragmentary picture of the Golgi apparatus. The method is uncertain and depends on various unknown factors. It resembles a staining reaction like Heidenhain's Iron-Haematoxylin but may be arbitrarily stopped too soon or carried to heavy overstaining. Unlike a Heidenhain preparation, however, no attempt is ever made to destain the silvered slide.

Since the standard Da Fano technique resembles a photographic silvering process such preparations may be carefully desilvered by use of a dilute solution of iodine. When this method is applied to spinal ganglion cells of the pig very striking results are produced. Its effectiveness is clearly demonstrated in over-stained preparations where formless deposits reveal, after desilvering, portions of a delicate reticulum. In preparations that would ordinarily be considered typical, the strands of the net are revealed as hollow canals, and short segments and granules become hollow vesicles. In all cases minute bodies resistant to destaining, and identified as mitochondria, are applied to the surface of the canals or vesicles.

It is strongly indicated by the results of this desilvering technique that the classical Golgi apparatus of nerve cells is a compound structure composed of (1) a canaliculan net (often fragmentary because of irregular shrinkage during fixation) and (2) a host of bead-like mitochondria lined up along the walls of the net.

61. PIGMENT-GENE PLEIOTROPY IN THE CARIBE-CUNA MOON-CHILD. Clyde E. Keeler, Georgia State College for Women, Milledgeville, Georgia. (Lantern; 15 min.)

Certain genes affecting melanic pigmentation also modify morphology, physiology and behavior in fish, birds and mammals. As a vertebrate phenomenon pigment-gene pleiotropy should be present in man. In outbred populations pleiotropic effects may be largely masked due to heterogeneous residual hereditary backgrounds, but the albinistic Caribe-Cuna Moon-Child variation is displayed on an inbred background that assures the detection of pleiotropy if it is present.

In the summer of 1950 thirty-seven Moon-Children and about two hundred controls were examined. The Moon-Child variation was found to be a mendelizing recessive, although shade of the heterozygote is often lighter than that of the average Indian.

Morphology: The skin lacks all pigment except sun-induced blotches, is dry, becomes wrinkled, is sensitive to infections at the site of wounds, abrasions and insect bites. Tissues of lips and about eyes become enlarged. The abnormally fine hair may change from white at birth through yellow to brown or red-brown in adults. Hair is present on forearms and lower limbs of all Moon-Children. Eyes tend to the oriental slit type, iris usually deep blue but dark at periphery and buff about pupil. Lateral nystagmus is universally present. Moon-Children are shorter and weigh less than their controls.

Physiology: Metabolic rate is probably altered, specific gravity is low, and ultraviolet spectrum analysis of urine reveals that albinos lack some metabolic end-product. Moon-Children usually carry infections of nose, head and throat. Strength is reduced, endurance lacking. Sexual development is slow with males undersexed.

Behavior: Mentality appears normal. (At least fourteen Moon-Children have competed in formal school.) Their gait and movements are slow. Their voices tend to be soft. They are said to be stubborn, and hot tempered. They seldom smile and when they do, it is but feebly. They lack facial expression. Moon-Children do not associate with each other. By persistence they may become leaders in their culture.

62. STAINING OF NUCLEIC ACIDS BY AZURE-PHTHALATE. Marion H. Himes and Martin H. Flax,¹ Columbia University. (Introduced by Arthur W. Pollister.)

A new dye, Azure-phthalate, histologically differentiates between ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Carnoy fixed tissues stained at pH 4 show chromosomes and interphase nuclei staining blue while nucleoli and cytoplasmic regions known to be rich in RNA stain red. After ribonuclease digestion, all the red staining of nucleic acid is selectively removed while deoxyribonuclease removes all substances that had stained blue. Prolonged deoxyribonuclease treatment not only removes DNA but also causes RNA staining to appear in the chromatin where previously there had been little or no indication of red staining. Direct inhibition of staining of chromatin RNA by DNA cannot account for this phenomenon since all the DNA is removed by deoxyribonuclease at a considerable time prior to the appearance of nuclear RNA staining. Tryptic

digestion of the tissue has the same effect as prolonged desoxyribonuclease treatment; in causing nuclear RNA to stain. It is suggested, therefore, that protein is so strongly bound to nuclear RNA that, under ordinary staining conditions, it is not displaced by the dye; when the protein is dissociated by special pretreatment, then the nuclear RNA can combine with the dye. It appears that the phosphoric acid groups of nucleolar and cytoplasmic RNA are less firmly bound to protein than are those of the chromatin, since pretreatment is not necessary for these nucleic acids to stain.

It has been observed that RNA stains blue when the dye is maintained at pH 3 and that this color becomes progressively more red as the dye solution is made more alkaline. Ribonucleic acids of different cell sites show this color change at different pH's. Nucleolar RNA of corn microsporocytes, for example, remains blue (at pH 3.5) while the cytoplasmic RNA has become reddish. These findings may provide a means of detecting qualitative differences between the ribonucleoproteins of different sites within the cell.

¹ Predoctoral fellow, National Cancer Institute, U. S. Public Health Service.

63. ULTRASONIC SOUNDS OF BATS, THEIR ACOUSTICAL DIMENSIONS AND BIOLOGICAL SIGNIFICANCE. Donald R. Griffin, Cornell University.

Live bats and portable electronic equipment, including cathode ray oscillograph, will demonstrate the production of inaudible, high frequency sounds and their principal properties: (1) high intensity (95 to 118 decibels above the standard reference level); (2) brief duration (1 to 3 milliseconds); (3) frequency modulation; (4) effects of cutting laryngeal nerves on the frequency and frequency modulation; (5) directional intensity pattern; and (6) the geometrical relations between a flying bat, the emitted pulse of ultrasonic sound, and the objects detected by hearing their echoes. Tape recordings will demonstrate bat pulses played back at reduced speed so as to lower all frequencies proportionately and provide a "literal translation" into the frequency range of human hearing.

64. ELECTRON MICROGRAPHS OF THIN-SECTIONED SPIROSTOMUM. Harold E. Finley, Department of Zoology, Howard University, Washington, D. C.

The National Bureau of Standards microsectioning method was used in a study of the ciliated protozoan *Spirostomum ambiguum*. Fine detail, which has not been resolved by ordinary light microscopy, has been photographed from sections prepared by that method. Some ultramicroscopic structures will be illustrated by means of electron micrographs.

65. ELECTRON MICROGRAPHS OF DIVIDING CELLS. H. W. Beams and T. C. Evans, The State University of Iowa.

Electron micrographs of the spindle fibers and centrosomes of different cells will be demonstrated. The effect of different fixatives upon the spindle will be shown.

66. MULTIPLE PORES AND CONTRACTILE VACUOLES IN PARAMECIUM. R. L. King, The State University of Iowa.

Observations have been made on the origin and fate of the vacuolar pores in *Paramecium aurelia*, *Paramecium caudatum* and *Paramecium multimicronucleatum*. Slides were made from a single pure line of each species by the nigrosin method. Dividing and non-dividing animals were studied on a single series of slides for each species. There is a tendency for the new pores formed during division to be multiple: in *P. aurelia*, 8.0%; in *P. caudatum*, 57.4%; and in *P. multimicronucleatum*; 65.6%. These seldom persist in *aurelia*, more frequently in *caudatum*, and most frequently in *multimicronucleatum*. In non-dividing *aurelia* the anterior pore is multiple in 1.4%, the posterior in none; in *caudatum* the anterior pore is multiple in 2.8%, the posterior in 1.8%; in *multimicronucleatum* the anterior pore is multiple in 34.7%, the posterior in 24.5%. In the two latter species they often function in association with an extra vacuole, and are passed on during division.

The extra contractile vacuoles so often seen in *Paramecium* are the result of this tendency to produce multiple pores and vacuoles at division; they have persisted from a previous division and are not precociously formed vacuoles in preparation for division.

67. THE ACQUISITION OF THYROXINE-SENSITIVITY BY TADPOLE TISSUES. William Etkin, The City College of New York.

Embryos of *Rana pipiens* were immersed in a 1:1,000,000 solution of thyroxine at various stages during early development and kept in the same solution continuously thereafter. Daily observations of metamorphic changes were made. It was found that embryos first exposed to thyroxine at the stage of the beginning of the formation of the opercular membrane (Pollister and Moore—stage 23) responded by a pattern of metamorphic changes essentially the same as that previously described for fully grown tadpoles in similar solutions. Animals exposed one, two or three days earlier than the stage of operculum formation showed no metamorphic changes until they reached the same stage of development as the above animals whereupon they displayed a similar pattern of metamorphic change. Control experiments in which thyroxine crystals were implanted into tail-bud embryos gave results similar to the immersion experiments. Therefore the delay in response to thyroxine shown by early embryos cannot be ascribed to impermeability of their skin. The evidence is interpreted as indicating that the tadpole tissues acquire sensitivity to thyroxine at about the time of the formation of the operculum. This acquisition of sensitivity appears to occur in all tissues at about the same time. The characteristic differentials in sensitivity seen in the tissues of the full-grown tadpoles are likewise present from the first appearance of sensitivity.

68. ELECTRON MICROGRAPHS OF CONNECTIVE TISSUE SPREAD PREPARATIONS SHOWING THE SUBMICROSCOPIC NETWORK OF FIBRILS. F. Wassermann, Argonne National Laboratory. (Introduced by Secretary.)

By spreading of pieces of subcutaneous fascia (mouse) on the slide, followed by immediate fixation, connective tissue was prepared for electron microscopy. A network of fibrils of widths from about 300 to 800 Å was demonstrated in both young and adult animals. The fibrils, about 10 times thinner than the finest silver fibers visible in the light microscope, are identical with those that compose fibers. This is evident when the elements of "frayed" fibers are compared in the electron micrographs with the solitary fibrils. The fibrils as such have been shown repeatedly by isolation from fibers and in tissue culture but their demonstration in situ as solitary structures forming a framework adds to the picture of connective tissue a regular submicroscopic component in the form of a micellar framework placed in the intermicellar material of the ground substance. The fibrils possess the periodic structure typical of collagen. Still finer strands of widths of about 50 to 100 Å can be seen best at magnifications of about 10,000 $\times$. They may be "protofibrils" of the kind previously described in tissue culture during fiber formation. Although the fibrils are structural units and formative elements of fibers, their regular presence suggests special function by chemical activity of these extracellular protein structures.

69. REGRESSION IN DENERVATED INJURED LIMBS OF AMBLYSTOMA. C. S. Thornton, and David Kraemer, Kenyon College.

Denervated limbs of Amblystoma larvae regress extensively when a localized area of the limb is injured by crushing or by perforation. Normally innervated control limbs quickly recover from similar injuries. In the majority of the cases (55 out of 67), regression was found to be more rapid and extensive at distal limb levels than at proximal ones. To stimulate regression in the denervated limb, the injury must include the internal limb tissues; a skin wound alone is ineffective as a stimulus to regression. Regression is not produced when the limb is denervated one week after being injured. Regeneration of the regressed limb, after reinnervation, occurs only when the digits have completely regressed. Reinnervated limbs which had regressed extensively do not regenerate if digits are still present.

70. CYSTIC LUNGS, A CONGENITAL ABNORMALITY ASSOCIATED WITH THE SHORT EAR GENE IN THE HOUSE MOUSE. Jean Smith, Ohio State University. (Introduced by E. L. Green.)

In the Sese-C1 strain of mice, which contains short and normal ear mice, a marked abnormality believed to be cystic lungs was found in all of a sample of 30 homozygous short ear mice (*sese*). A lesser degree of the condition was present in all of 31 heterozygous normal ear mice (*Sese*). The cysts which appear as small liquid-filled blebs were found throughout the substance of the lungs in the *sese* mice, but chiefly at the periphery in the *Sese* mice. They occurred in all ages examined from 0 to 100 days. Longevity does not appear to be affected. This strain

had been inbred by brother-sister matings with forced heterozygosity of the short ear gene and was at the time of the examination in its 24th and 25th generation. Whether this is a new manifestation of the short ear gene or of genes closely linked with it cannot now be determined. An equally inbred strain Sese-Ab, also containing *Sese* and *sese* but of different origin, was examined and no similar blebs were found.

71. THE DEVELOPMENT IN VITRO OF GIANT CELLS FROM HUMAN PERIPHERAL BLOOD.
M. Goldstein, Western Reserve University.

Tissue cultures are prepared on cellophane in D-5 Canel flasks. The buffy coat which is formed on centrifugation of citrated whole blood is reclothed and then cut into small pieces and placed on the cellophane. The proliferating leucocytes have been fixed at various periods after incubation in a given medium. The development of multinucleated giant cells that are histologically similar to those seen in foreign body reactions and in tuberculosis.

72. THE ORIGIN AND DEVELOPMENT OF THE RESPIRATORY LABYRINTH IN THE PARADISE FISH, *MACROPODUS OPERCULARIS*. S. Ito, Western Reserve University.

This study of the accessory respiratory organ was made to determine the origin and to follow the development by means of serial sections of various sizes and ages of developing fish.

73. THE SIGNIFICANCE OF MITOCHONDRIA IN EMBRYONIC CHICK PANCREAS. R. D. Shieman, Western Reserve University.

This work involves a study of the mitochondria and zymogen granules of the pancreas of chick embryos in an effort to determine at what stage of development the pancreas secretes its enzymes.

Demonstrations by Motion Pictures

Section C

74. RESUMPTION OF HEART-BEAT IN CHICK EMBRYOS FROZEN IN LIQUID NITROGEN.
B. J. Luyet and F. Gonzales, Saint Louis University. (12 min.)

The 40-hour chick embryo was found to be an excellent material to show the spontaneous resumption of some activities in an organism which has been congealed. Its bulk is small enough to permit a rapid cooling and rewarming, it can support a slight dehydration, and it has a well observable heart-beat.

The preparation and treatment of the embryos include: (1) Cutting the membranes which maintain the embryo proper on the yolk; (2) Transporting the embryo from the egg to a thin mica support; (3) Dehydrating it in a hypertonic solution of ethylene glycol; (4) Dehydrating it further by a short exposure to the air, and reversing the embryo on the mica support to permit a similar dehydration

of the underside; (5) Immersing it in liquid nitrogen for rapid cooling; (6) Immersing it in physiological saline at 40°C. for rapid rewarming.

In the embryos dehydrated properly, and frozen and thawed rapidly, the heart resumes its beating and maintains it for several hours, though the beats are usually less intense than in the normal untreated embryo and often have an irregular rhythm.

75. SURVIVAL OF VINEGAR "EELS" AFTER CONGELATION IN LIQUID NITROGEN. B. J. Luyet and P. M. Gehenio, Saint Louis University and Biodynamica Research Laboratory, Normandy, Missouri. (12 min.)

The experiments to be described here were based on the idea (1) that organisms may survive congelation at very low temperatures if they are cooled and rewarmed with sufficient rapidity to forestall crystallization in the temperature range in which crystallization normally occurs, that is, between zero and a few tens of degrees below zero, and (2) that a reduction of water content helps to prevent crystallization.

The procedure is essentially as follows: (1) A drop of vinegar, heavily infested with *Anguillula aceti*, is deposited on a thin piece of mica under the stereoscopic microscope. (2) The vinegar in that drop is blotted off with tapered strips of filter paper, the animals being thereby gathered in a mass in the center. (3) This preparation is kept for 24 hours in an incompletely closed moist chamber where the worms undergo a slight dehydration. (4) Next it is immersed in liquid nitrogen (where it may be kept for a day). (5) It is then rewarmed rapidly by sudden immersion in sterile vinegar at 30°C.

The rewarmed worms resume motion slowly: one or two minutes generally elapse before they begin to move and then their extremities only are moving; after 5 to 10 minutes the motility has extended to the entire body, but the movements are still slow and somewhat cramped; after an hour or two motion is about normal in a large percentage of the animals.

76. WHITE THROMBO-EMBOLISM IN THE TRANSILLUMINATED CHEEK POUCH OF THE HAMSTER FOLLOWING TRAUMA, ANTICOAGULANT THERAPY, INFECTION AND MALIGNANT NEOPLASIA. Brenton R. Lutz, George P. Fulton and Robert P. Akers, Department of Biology, Boston University. (15 min.)

White thrombus formation and fragmentation resulting in circulating emboli were cinephotomicrographed at magnifications of 200 to 1200 X in the transilluminated cheek pouch of the hamster following experimental endothelial trauma, during general infection and in advanced malignant neoplasia. Large platelet thrombi were produced in venules by vascular occlusion and by administration of urethane twice weekly for 3 months. Thrombus fragmentation resulted in emboli in the arterioles of the traumatized pouch and in the opposite pouch, at times blocking arterioles and capillaries. Platelet thrombi and emboli were produced by inserting a microneedle through the venule wall and traumatizing the endothelium on the opposite side or by strong stimulation with a micro-electrode. Dicumarol prevented platelet thrombo-embolism, but produced leucocytic coatings

on the endothelium and leucocytic thrombo-embolism. Heparin prevented platelet thrombosis, but produced platelet embolism and leucocytic coatings.

Mixed platelet and leucocytic coatings and emboli were found extensively during infection with *S. aureus*, and in hamsters with advanced cheek pouch transplants of methylcholanthrene-induced sarcoma. The emboli sometimes plugged small arteries and capillaries.

Sludged blood was not observed during any of the experimental procedures. In some hamsters, the erythrocytes in some vessels circulated in groups separated by plasma resulting in a type of flow which might be mistaken for sludged blood if examined by reflected light at low magnifications. However, the cells in the groups were not adherent to each other, moved individually within the group, entered the capillaries singly, and were readily separated by probing with a microneedle.

77. SERIAL SARCOMA TRANSPLANTATION IN THE HAMSTER CHEEK POUCH, AND THE EFFECT OF ADVANCED NEOPLASIA ON THE SMALL BLOOD VESSELS. Brenton R. Lutz, George P. Fulton, Donald I. Patt, Alfred H. Handler and Dean F. Stevens, Department of Biology, Boston University. (15 min.)

The cheek pouch of the hamster, *Mesocricetus auratus*, was used in a quantitative study of the effect of the number of passages on the growth characteristics of a new malignant methylcholanthrene-induced sarcoma. Pieces measuring 0.5 cm were introduced into the vascular bed between the epithelial layers of the everted cheek pouch. The transplant growths were free and symmetrical. Their sizes were measured with an ocular micrometer and vertical scale at 48 and 72 hour intervals for 30 to 45 days. Average first generation transplants, after a lag of 12 to 15 days, grew exponentially to 172 cmm during the first 20 days. Average second generation transplants (second cheek pouch passage) showed a lag of only 6 to 12 days and attained 312 cmm during the first 20 days. Average third generation transplants with a similar lag, grew to 430 cmm in the first 20 days. The color moving pictures show the technique of transplantation, the gross appearance of the tumors from the beginning to ulceration, and the microscopic details of the original tumor, cheek pouch transplants and metastases. Transilluminated early preparations show details of beginning vascularization. The transilluminated small blood vessels of the opposite pouch at magnifications of 200-1200 X, in advanced neoplasia, show white thrombo-embolism. Mixed platelet and leucocytic coatings, thrombi, and fragmentation into emboli are shown. No true blood sludge was found.

78. PRESERVATION OF RED CELLS IN BLOOD FROZEN IN LIQUID NITROGEN. B. Luyet and L. Menz, Saint Louis University. (10 min.)

The congelation of blood being a classical means of producing hemolysis in the laboratory, it was of particular interest to see if the method of ultra-rapid cooling and rewarming would permit the congelation of blood without the destruction of the erythrocytes.

The procedure was the following: (1) A drop of oxalated ox blood was deposited on a thin mica support. (2) This preparation was immersed in liquid nitrogen (where it sometimes stayed for hours). (3) It was then shaken vigorously in a small amount of an isotonic saline solution at 40°C. for rapid rewarming. Several drops were shaken in the same rewarming bath to increase its concentration of blood cells.

The rapidly cooled and rewarmed blood gives an opaque suspension, while blood cooled slowly (in the vapor above liquid nitrogen) or/and rewarmed slowly (in the air) gives a transparent solution of hemoglobin. The fact that the red cells are intact after ultra-rapid cooling and rewarming is confirmed by microscopic examination.

79. INTRINSIC CONTRACTILITY IN THE EMBRYONIC RAT HEART. E. K. Hall, Department of Anatomy, University of Louisville School of Medicine.¹

The hearts of 12-day and 13-day rat embryos were excised and allowed to remain in Ringer's solution at 30–32°C. Such hearts consist of a large atrial chamber only very slightly subdivided by the developing septum primum, and a ventricular chamber in which the interventricular septum is only beginning to separate future right and left ventricles. These hearts, after a few minutes, begin to beat regularly at the rate of 121.3/min. (average of 89 hearts). This rate of contraction is almost the same as that previously observed in the 13-day embryos before excision of the heart (120.7/min.), and is faster than that previously observed in the intact 12-day embryos (85.8/min.). The heart was then divided at the atrio-ventricular junction. After a few minutes, the two fragments begin to contract regularly, the atrial fragment at the rate of 141.4/min., the ventricular fragment at 72.7/min. (averages of the 178 fragments). Many of these fragments continue to beat for hours; 75 of the 178 fragments were beating the following day, and of these, 22 were observed to beat 24 hours after removal of the heart from the embryo. The longest period of survival was 29 hours in two ventricular fragments.

¹ Supported by a Grant-in-Aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council.

80. LIFE CYCLE OF *DIPHYLLOBOTHRIUM LATUM* (BROAD FISH TAPEWORM). M. S. Ferguson, U. S. Public Health Service, Atlanta, Ga. (30 min.)

Stages in the life cycle of the fish tapeworm are shown starting with the living adult tapeworm from a human intestine. The eggs are shown hatching, coracidium; oncosphere; intermediate host, *Diaptomus*; formation of proceroid. The life cycle continues with dissection of muscle of fish; transfer to final host, development of adult tapeworm.

Animal Biology and Sociobiology

Section A

81. SOCIAL BEHAVIOR AND ORGANIZATION IN THE DOG. J. P. Scott and John L. Fuller, Roscoe B. Jackson Memorial Laboratory. (Motion picture; 15 min.)

Classifying individuals by age and by sex, six major social relationships may be developed. The patterns of social behavior seen among dogs, and particularly the course of development of these patterns, tends to intensify the relationship between young and young, with the result that the litter becomes the most important natural social group. Within this group all major types of social behavior may be seen. In early life contactual behavior and playful fighting are important; later allelomimetic and investigative behavior appear. Sexual and epimeletic behavior are not important previous to sexual maturity. As a species, adult dogs predominantly exhibit allelomimetic, conflict, and investigative behavior, although other types may be prominent at special times. In organization, the litter group is strongly coordinated by allelomimetic behavior, but little leadership is developed. Strong systems of dominance may be present. The social relationship developed between pet dogs and man appears to be similar to the parent-child relationship of human beings, but not as closely similar to the adult-young relationship of dogs. Some comparisons with wolves are made.

82. THE EFFECT OF X IRRADIATION ON FIGHTING BEHAVIOR IN MALE MICE. Howard H. Vogel, Jr., Argonne National Laboratory. (Lantern; 16 mm motion picture; 15 min.)

These experiments were carried out to test the effects of chronic X irradiation upon fighting behavior. Male mice (5 weeks old) of three strains, C-57 black, C-3H agouti, and CF-1, were kept in isolated cages and were trained to fight in a multiple-escape pen. Six males were trained to become aggressive, whereas, under the conditions of the experiment, fighting was inhibited in their daily opponents.

After the trained fighters had defeated a dozen other males of their strain, they were irradiated daily with 50 r X radiation, 5 days a week, and fighting was continued daily. The body weights of the irradiated fighting mice were compared with those of nonirradiated controls over a period of approximately fifteen weeks. The accumulated X-ray dosage given to each of the six experimental mice at the time all fighting ceased and at the time of death are given.

The effects of X irradiation upon fighting behavior in male mice were cumulative. Each fighting mouse retained its characteristic method of attack up to the day when all fighting ceased. There was a definite threshold after which no fighting occurred, but before this threshold the irradiated fighters defeated their daily opponents. The aggressiveness of the attacks decreased as the cumulative X-ray dose increased. The conditioning that the mice had received was strong and it took a large dosage (1200 to more than 2000 r) to eliminate it completely. All signs of fighting were not suppressed until shortly before the death of the irradiated animal.

83. HEREDITARY DIFFERENCES IN SOCIAL BEHAVIOR AND SOCIAL ORGANIZATION BETWEEN DBA AND C57 BLACK INBRED STRAINS OF THE HOUSE MOUSE (*MUS MUSCULUS*). John B. Calhoun, Division of Behavior Studies, Hamilton Station, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. (Lantern; 15 min.)

This paper describes a research program designed to analyze the role of the determinants (heredity, environment and culture) of social organization. Two identical environments were constructed. In each, food, water, and nesting material were continuously and abundantly available at a single locality. The only variable was related to availability of harborage. Successive groups of nest boxes were further removed from the source of food, etc. One pregnant *dba* mouse was introduced into one of these environments, and a pregnant C57 in the other. These mothers, whose only past experience was of inhabiting a 6 × 12 inch breeding box, were removed as soon as the young were of weaning age.

These two strains of mice differed in most, but not all of their behavior. The *dba* mice were quite stereotyped in their behavior; it was only very gradually, if at all, that new or modified behavior patterns were assumed, but once assumed a behavior pattern was quite invariable. On the other hand, the C57 mice were quite labile in their behavior; they readily adjusted to new situations as they arose. These characteristics are expressed in relation to both the physical and social aspects of the environment. Among the *dba* mice each encounter between two individuals usually was followed by an exhibition of social position—usually by fighting. This developed a rigid hierarchy. The C57 mice rarely fought. Two individuals seeking the same goal will alternately relinquish priority to it. This alternate relinquishing of priority essentially replaces the hierarchial organization seen in *dba* mice.

84. AGE AND STRAIN DIFFERENCES IN RESPONSE TO ELECTRO-CONVULSIVE-STIMULATION (ECS) IN THE MOUSE. Alfred Coulombre, Roscoe B. Jackson Memorial Laboratory. (Introduced by John L. Fuller.) (Motion picture; 15 min.)

Convulsions similar to those used in shock therapy of human patients were induced in mice of various ages and of several strains by a 1/8 second, sixty cycle shock applied through the ears. Death during convulsion does not occur until between twelve and twenty days of age. Thereafter DBA² mice show significantly higher death incidence during tonic convulsion than do C57 Bl⁶ mice or C57 Bl⁶ × DBA² hybrids. The postural pattern characteristic of electrically induced tonic convulsion changes with age. Newborn mice up to four days of age show a lateral extension of all limbs lasting from one to three seconds. Those between four and six days exhibit forelimb extension cephalad, and hind limb flexion. Between six and eleven days shock elicits forelimb extension posteriad, and hind limb flexion. At about twelve days the definitive postural pattern appears. This consists of forelimb flexion, and hind limb extension posteriad. These changes at definite ages suggest a step-wise process of maturation in the nervous system.

Mice of the C-Scott strain show marked clonic activity in response to ECS up to about twelve days when the definitive tonic postural pattern makes its appearance. This strain, unlike others studied, exhibits slow, blind, circling running in the post-tonic convulsive phase.

85. THE LEVEL OF THYROID ACTIVITY IN THE GUINEA PIG.¹ Mina MacNair Brown, Roy R. Peterson, Barbara Rayner and William C. Young, University of Kansas. (Lantern; 7 min.)

Oxygen consumption, heart rate and level of protein-bound serum iodine (PBI) were used as indices of thyroid activity in guinea pigs.

In 30 normal males oxygen consumption averaged 54.7 cc per 100 grams body weight per hour, heart rate 261 beats per minute, and PBI 2.2 percent (1.0 to 3.4). Comparable values for 23 females were 58.9, 265 and 2.3 (0.6 to 3.5).

Following surgical thyroidectomy oxygen consumption was 48.7 in 5 males and 52.2 in 4 females, heart rate 236 in the males and 251 in the females, PBI 1.5 (1.0 to 2.0) and 1.7 (1.1 to 2.0), respectively. For the three indices of thyroid activity therefore the decreases were small. This is believed to indicate that the thyroid of the guinea pig maintained at 70 to 75 degrees F. functions at a low level.

Crystalline thyroxine was administered to 6 males (up to 0.05 mg per day) and 10 females (100 mg every 4 days or 150 mg every 3 days). Oxygen consumption was 80.1 in the males and 93.5 in the females, heart rate 33 in both sexes, and PBI 5.4 (3.7 to 8.4) in the males, and 8.4 (6.2 to 11.3) in the females. The changes following thyroxine administration are regarded as a check on methods. They indicate moreover that the guinea pig is responsive to thyroid hormone despite the probability that under the conditions in this laboratory the thyroid does not function at a high level.

¹ Aided by grants from the U. S. Public Health Service.

86. SEX DRIVE AND THE LEVEL OF THE THYROID ACTIVITY IN THE MALE GUINEA PIG.¹ William C. Young, Roy R. Peterson, Barbara Rayner and Mina McNair Brown, University of Kansas. (Lantern; 8 min.)

The hypothesis of a relationship between strength of sex drive and the thyroid was tested in 20 animals. Strength of drive during a pretreatment period was determined from 10 tests of each animal. Five were then thyroidectomized, 6 received up to 0.05 mg crystalline thyroxine daily for 5 months and 9 were controls. Each was given 10 tests late in the post-treatment period.

The average change in drive was as follows: Controls, — 10% (— 1.6% to — 25.6%); thyroidectomized males, — 9.9% (+ 14.3% to — 26.4%); thyroxine-treated males, — 9.7% (+ 19.1% to — 28.3%). The differences are not significant.

The average scores of the second 10 tests were as follows: Controls, 7.162 (5.440 to 9.238); thyroidectomized males, 7.600 (6.970 to 8.620); thyroxine-treated males, 6.749 (4.224 to 9.700). Average oxygen consumption (cc/100 gr/hr) was 53.0 (46.7 to 62.5), 46.7 (42.1 to 68.0) and 80.1 (70.1 to 94.0), respectively. Average heart rates were 245 (213 to 285), 236 (202 to 274) and 332 (310 to 343), respectively. Serum protein-bound iodine (γ percent) averaged 2.1 (1.5 to 2.4), 1.5 (1.0 to 2.0), and 5.4 (3.7 to 8.4), respectively.

Clearly, differences in behavior were not proportional to differences in indices of thyroid activity. It is also evident that thyroxine at the dosage given did not

affect the apparently natural decrease in strength of drive. We conclude that within the limits established experimentally, variations in amount of thyroid hormone do not influence strength of sex drive.

¹ Aided by grants from the U. S. Public Health Service.

87. SEXUAL BEHAVIOR IN THE GUPPY. Eugenie Clark and Lester R. Aronson, The American Museum of Natural History. (Lantern; 15 min.)

One of the most striking features of the sexual behavior in the guppy *Lebistes reticulatus* is the manner in which the male pursues the female and the frequency with which he jabs at her genital region with a momentary thrust of the gonopodium. Previous reports assumed that this behavior represented copulation and sperm transfer. By means of genital smears, the present study shows that these thrusts never resulted in insemination. In effective copulations which occur infrequently and are rarely seen, there is a more extensive contact between the male and female which may last as long as 2.4 seconds. At this time the female remains quiescent and almost stationary, suggesting a definite receptive stance. Just prior to copulation the body of the male often becomes tense and curves in an "S"-like position and while in this state he moves to the side of the stationary female and thrusts his gonopodium at her genital opening.

Experiments designed to test the function of the gonopodium by means of partial amputations indicate the importance of the distal portions of rays 3, 4, and 5 for effective copulation. However, removal of the gonopodial hood does not prevent insemination. The tip of the hood contains an extensive nerve plexus which suggests that it may serve in a sensory capacity. In *Platypoecilus* and *Xiphophorus* the tip of the gonopodium actually hooks on to the female and thus serves as a holdfast organ. However, in the guppy the tip is less specialized and contact is maintained by the male who actively swims and pushes the gonopodium against the stationary female.

88. FACTORS INFLUENCING EMBRYONIC SURVIVAL OUTSIDE OF THE MOUTH OF THE ORAL INCUBATING FISH, *TILAPIA MACROCEPHALA*. Evelyn S. Shaw, The American Museum of Natural History, New York University, and Newark College, Rutgers University. (Introduced by C. M. Breder, Jr.) (Lantern; 15 min.)

Tilapia macrocephala, the oral incubating fish, breeds readily in fresh water aquaria. However, if the embryos are removed from the mouth prior to the third day of development they do not survive in fresh water even in the same tank with the incubating parent.

Sea water diluted with fresh water to various salinities proved to be the most effective medium for extra-oral development. The survival rate rose with increasing salinity, maximum survival being attained in solutions containing 40-60% of sea water. The survival rate decreases slightly at higher salinities. Eggs placed in this optimum medium soon after fertilization grew and developed normally.

Preliminary results indicate that dilute solutions of methylene blue chloride and a bactericidal solution containing four salts were somewhat less effective in maintaining the embryos.

When fresh water was continuously circulated over the eggs for six days, sporadic survival was obtained with eggs subjected to the flow in the early cleavage stages, but more consistent and higher survival rates were achieved with embryos in later stages (e.g., blastula) of development. Controls in sea water solutions developed normally and secondary controls in non-circulating fresh water did not survive.

In an examination of the adult's oral cavity, two glands of unusual nature and of an unknown function were noted. Histological studies indicated that they are aggregates of large mucus secreting cells. Caution of these glands did not decrease embryonic survival in the mouth.

The relationship of these findings to the ecology of the species will be discussed.

89. ATTENTIVE BEHAVIOR OF THE HOUSE WREN, *TROGLODYTES AEDON*. S. Charles Kendeigh, University of Illinois. (Lantern; 15 min.)

Thermocouples and itographs automatically recorded all trips of adults to the nest. Full incubation behavior develops gradually. Whether or not a sixth egg is laid is determined two or three days earlier and is associated with changes in behavior. Only the female incubates, in average attentive periods of 12.1 minutes with intervening inattentive periods of 8.5 minutes. Total attentive time on eggs decreases at high air temperatures. Length of attentive periods is influenced by time to digest a stomach full of food, the length of inattentive periods by the time required to fill it. Both adults feed the young for 15 days in the nest during which time the average daily rate increases as a sigmoid curve from 184 to 256. The total number of feedings increases with the size of the brood but the rate per young bird decreases from 115 per day with one present to 50 when six occur. The larger broods stayed one day longer before flying than did the smaller broods. There is a pronounced daily rhythm, with attentiveness reaching its lowest level in early afternoon. The female's daily activity begins with sunrise and ends shortly before sunset, but the male is active several minutes earlier in the morning and later in the evening. Some recordings indicate that the female's periods of quiet and restlessness at night simulate periods of attentiveness and inattentiveness during the day. It is evident that the bird's behavior is influenced by its physiological state and by environmental conditions.

90. TERRITORY AND NESTING BEHAVIOR IN NESTING CLIFF SWALLOWS. John T. Emlen, Jr., University of Wisconsin. (Lantern; 15 min.)

Cliff swallows (*Petrochelidon pyrrhonota albifrons*) studied at Jackson Hole, Wyoming showed a closely integrated type of flock behavior at their nesting colonies, on their foraging grounds and on loafing perches near the colonies. Flock formations and flock densities indicated a strong mutual attraction between all swallows in the region whether or not they were members of the same colony. This positive force of social attraction was balanced by a clearly defined negative

force of individual intolerance which kept all birds within the group at a distance of four inches or more from each other when at rest. Individual rights within this limit were usually respected by other birds; the occasional intruder inevitably precipitated aggression and readjustment. When associated with a fixed site at the nesting colony, this individual intolerance response was indistinguishable from territorial defense as classically described. Experiments revealed that the important recognition features of individual territories were restricted to the immediate vicinity of the nest opening.

91. ADDITIONAL OBSERVATIONS ON THE SAGE GROUSE. John W. Scott, University of Wyoming. (16 mm motion picture; 15 min.)

Characteristic behavior of the sage grouse during the mating cycle has been described, 1942 and 1950. Annual observations on one area, 1940-1950, have disclosed additional facts. The population has fluctuated, with general tendency downward. The strutting area remains about the same size, but has shifted northward, possibly due to increasing visitors, or because some large cocks were killed on barbed wire fence crossing southern part of area. All principal mating spots were located formerly south of the creek; in 1950, none remained there. An orderly, well-organized mating spot requires several years to develop. For example, after an eagle disperses the flock, as the birds wander back on the area, a vigorous cock may attract a small group of anxious hens and succeed in mating one or two, before the regular mating spots are occupied. Such a cock acquires prestige by repetition and by fighting off other cocks. The characteristic social organization remains as previously described. But shifting of mating spots to new locations tends to create confusion and uncertainty. Consequently behavior appears less stereotyped than in earlier years. The mating season lasts about seven weeks; the mating peak lasts from 8 to 13 days, sometimes split into two periods by severe weather. The season is also related to mean temperature, and interference by the eagle predator frequently prolongs the peak period. One albino hen and one albino cock were seen.

92. SOME BASIC PSYCHOLOGICAL AND NEURAL MECHANISMS OF SOCIAL BEHAVIOR IN CHICKS. N. E. Collias, University of Wisconsin. (Lantern; 8 min.)

The clucking of a broody hen inhibits the distress calls of her chicks, and also causes them to emit notes of very different character which could well be termed "pleasure notes." Tests with phonograph records have shown clucks to have an optimal quality, pitch, rate, and loudness; effective variations have a wide range. The characteristic distress calls may be given repeatedly under many conditions of distress that at the same time inhibit pleasure notes; isolation from companions, cold, hunger, thirst, pain, restraint, or approach of a large object. Conversely, the opposite situations as a rule lead to pleasure notes and inhibit distress calls. These and other facts suggest the existence of two anti-theoretical neural systems, balanced against each other, and corresponding to what in man would be called security-insecurity feelings and responses.

In general, a chick moves toward stimulus situations that cause it to emit pleasure notes, and away from such situations as bring about distress calls. What

a chick does in a complex social situation depends on the balance of the total external and internal conditions.

The social responses of baby chicks have many properties in common with the central nervous system including latency, rhythmicity, fluctuating threshold, summation, afterdischarge, specific fatigue, and inhibition.

Decerebrate chicks will not move toward a clucking or retreating object, but if the basal portions (paleostriata) of the cerebral hemispheres be left intact while removing the rest of the cerebrum, the social responses remain almost normal for a few days or more.

93. THE SOCIALIZATION OF CHICKS. N. E. Collias, University of Wisconsin, (Lantern; 7 min.)

Upon hatching away from a hen chicks give characteristic distress calls caused by loss of contact with the egg shell and by cooling of the moist down. They pay no attention to mounted hens. Within one-half hour most chicks respond to clucks or to moving objects by cessation of distress calls, and, as soon as they can walk, by moving toward the stimulus. At first the response is slow, but after some minutes of repeated exposure a chick responds much more rapidly and over greater distances. At the same time the need for the operative social stimulus is increased as indicated by more frequent distress calls between stimulus periods. The food guidance, warm contact and protection which a hen gives her chicks no doubt strengthen the family bond. Increasing social discrimination is indicated by attachment of chicks to the specific hen that rears them, and by reduced responsiveness to artificial stimuli.

Early social plasticity is indicated by considerable decline in response of 10-day old chicks on first exposure to mechanically recorded clucks, and in their lesser tendency to follow a large, retreating object, as compared with chicks in the first few days of life.

Recently hatched chicks do not readily approach one another until after experiencing some minutes of bodily contact, following which they rapidly come together when separated over short distances. Chicks isolated from hens and other chicks until 10 days of age are less gregarious than normals.

The contagiousness of chick behavior facilitates aggregation and socialization. Sometimes certain individuals quite consistently lead stray individuals back to the group. Some individuals are characteristically laggards in certain of their social responses.

Endocrinology

Section B

94. FURTHER EVIDENCE OF FACILITATION OF COMB RESPONSE TO ANDROGEN BY THYROID HORMONE. David M. Morris, North Texas State College. (Lantern; 15 min.)

The influence of thyroid hormone on comb growth in the White Leghorn fowl was studied by varying the level of thyroid hormone and/or androgen by one

or a combination of the following procedures: thyroidectomy, testosterone, injection, castration, and thiouracil administration. The response of the comb to both endogenous and exogenous androgen seemed to be proportional to the thyroid activity as evidenced by the following results: (1) comb growth in totally and partially thyroidectomized birds was directly related to the amount of thyroid tissue present, (2) testosterone propionate was less effective in augmenting comb growth in athyroidic than in normal birds, (3) the retarded comb growth observed in gonadectomized birds was accentuated in thyroidectomized capons, (4) thiouracil caused a significant inhibition of comb growth and reduced the response of the comb to administered androgen. Analysis of the data suggests a synergistic relationship between the androgen and thyroid hormone in promoting comb growth.

95. THE EFFECT OF MAMMALIAN THYROID POWDER AND THIOURACIL ON GROWTH RATES AND ON THE DIFFERENTIATION OF THE GONOPOD IN *LEBISTES RETICULATUS*. Arthur F. Hopper, Rutgers University. (Lantern; 15 min.)

Young *Lebistes reticulatus* raised in a 0.01% concentration of desiccated mammalian thyroid powder for a period of from 3 to 6 months showed a marked increase in growth rate over control fish from the same brood. The response of the females was greater than that of the males, the average size of all thyroid-treated females being about 50% larger than the average size of all control fish. Thyroid-treated males averaged about 30% larger than control males. Raising young fish in a 0.03% concentration of thiouracil resulted in about a 20% decrease in growth rate in the treated fish.

The first indication of gonopod differentiation in control fish appeared 78 days after birth, while thyroid-treated males began the maturation of the gonopod as early as 18 days after birth and thiouracil-treated males delayed gonopod-differentiation until 130 days after birth. Gonopodia of both the thyroid-treated and thiouracil-treated males were abnormal.

Histological examination of the thyroid glands of thiouracil-treated fish revealed a marked hyperplasia of the gland with follicles present from the anterior portions of the lower jaw as far posterior as the stomach. Metastases to the gill arches and to the stomach were observed. Individual follicles were generally small with cuboidal epithelium and scanty colloid.

96. BIOLOGICAL ACTIVITY OF 17-VINYL TESTOSTERONE. C. H. Howell and N. W. Antonchak, Ciba Pharmaceutical Products, Summit, N. J. (Introduced by L. A. Stauber.) (Lantern; 15 min.)

The androgenic activity of 17-vinyl testosterone was tested in normal and castrated immature rats. A 100 percent increase in seminal vesicle weight was induced in 72 hours following one subcutaneous injection of 5.0 mg. In contrast, one subcutaneous injection of 5.0 mg did not increase uterine weight in normal or spayed female rats. However, after 15 daily injections of 5.0 mg, ovarian weight of young rats averaged only 15.1 mg as compared with 24.2 mg in litter-mate controls. This steroid had a 2 plus progestational activity at 7.5 and 10.0 mg

total dosages when tested in estrogen pretreated immature rabbits but failed to maintain adrenalectomized immature rats with 2.0 mg daily dosages. Vinyl testosterone antagonizes the increase in adrenal weight which follows unilateral adrenalectomy and exercises an anti-insulin action in fasting mice. 17-vinyl testosterone has androgenic and progestational activity but lacks estrogenic or life maintaining activity at the dosages tested. This steroid has an anti-ACTH and anti-insulin action.

97. THE INHIBITION OF UTERINE GROWTH BY A SULFHYDRYL INHIBITOR. H. A. Salhanick, Melvin H. Farmelant, Thomas C. Smith, and Frederick L. Hisaw, Harvard University. (Lantern; 15 min.)

The rapid and quantitative response of the uterus to estrogenic stimulation makes this organ ideal for studying the metabolic and enzymatic processes involved in growth. This unique response has already been utilized to demonstrate that competitive antagonists to folic acid will prevent the rapid growth observed after estrogen treatment.

The role of sulfhydryl enzymes in growth and metabolism has been extensively investigated. It was the purpose of this investigation to determine whether free sulfhydryl groups are necessary for the estrogenic action on the uterus.

Para-chloromercuribenzoic acid (PCMB) was chosen as the inhibitor because of its apparently greater specificity for sulfhydryl groups than the other known inhibitors. One-day-old chicks received injections of PCMB prior to and along with stilbestrol injections. Both the wet and dry weights of the oviducts of chicks treated with inhibitor and estrogen were less than the estrogen-treated controls, and increasing doses of PCMB caused proportionately greater inhibition. Similar experiments performed on the uteri of adult ovariectomized mice and rats gave comparable results. Furthermore, administration of 2:3-dimercaptopropanol (B.A.L.) prevented the inhibition caused by PCMB.

Since it has been demonstrated by many workers that most vitamin-antagonists and toxic organic compounds do not affect uterine growth and since complete starvation did not prevent uterine growth in rats treated with estradiol, it is concluded that PCMB acted *in vivo* as it does *in vitro*. This indicates that free sulfhydryl-containing molecules or sulfhydryl enzymes are necessary for estrogen-induced uterine growth.

98. A COMPARISON OF REPRODUCTIVE POTENTIAL OF TWO RAT POPULATIONS. David E. Davis, The Johns Hopkins School of Hygiene and Public Health. (Lantern; 10 min.)

The discovery that the reason Norway rats (*Rattus norvegicus*) living in the city were significantly larger than rats on a farm was due to a deficient diet on the farm suggested a comparison of the growth and reproductive rates. Hence, a comparison of size at attainment of several growth characters and of production of young was made. It was found that city rats grew faster and attained reproductive maturity earlier than did the farm rats.

Pregnancy was correlated positively with size in both populations. The crude incidence of pregnancy was not significantly different in the two places but the incidence of lactation was significantly higher in the city (4.5 per year) than on the farm (2.2 per year). Also the mean number of embryos was significantly higher in the city (10.1) than on the farm (8.2). Allowing for a 20% mortality at and after birth, the city females weaned about $8.1 \times 4.5 = 36.0$ young per year while the farm females weaned about $6.2 \times 2.2 = 13.6$ young.

Using these values and a knowledge of population changes it is estimated that the death rate in the city was about 14.4 and on the farm was about 5.6 deaths per rat per year.

99. PROGESTERONE LEVELS IN THE EWE. G. M. Neher and M. X. Zarrow, Department of Biological Sciences, Purdue University. (Lantern; 10 min.)

Progesterone levels were determined in the blood serum of the ewe by the Hooker-Forbes assay procedure. According to these authors the test will detect 0.0002 micrograms of progesterone when injected into the lumen of the uterus of a castrated mouse. Serial dilutions of each sample were prepared and tested in a minimum of two mice for each concentration. A total of 7 adult ewes were studied throughout pregnancy and immediately postpartum. Length of pregnancy was dated from the day after mating and confirmed by the lambing date. This was found to average 147 days.

A concentration of 2 μ g of progesterone per ml of blood serum was found at estrus. This level increased rapidly during the first part of pregnancy reaching a concentration of 6 μ g per ml by the 22nd to the 40th day. No further changes in the amount of progesterone were noted until about the 100th day. At this time the concentration began increasing again and reached a peak of 10 to 12 μ g per ml at parturition. Examination of the serum at 10 hours postpartum revealed no appreciable change in progesterone, but by the 36th hour postpartum a 50 per cent drop was observed. At 10 days postpartum the progesterone had decreased to approximately 2 μ g per ml.

General Physiology

Section A

100. RESPIRATORY MOVEMENTS, OXYGEN CONSUMPTION AND BRAIN METABOLISM IN TEMPERATURE ACCLIMATIZATION OF GOLDFISH. John A. Freeman, Newcomb College of Tulane University. (Lantern; 15 min.)

During acclimatization to lowered temperatures, poikilothermal animals exhibit alterations in lethal temperatures, in activity and in total metabolism which appear to be adaptive. Respiratory movements have been observed as activities which are normal and necessary to the goldfish; oxygen consumption has been used as a measure of total metabolic activity; and brain tissue metabolism has

been studied as a possible factor in maintenance of respiratory and other activities and in their alterations during acclimatization. Rates of respiratory movements at acclimatization temperatures between 4° and 27° rise rapidly with temperature but are the same at 37.5° as at 27°. Oxygen consumption also rises between 12° and 27° but is lower at 37.5°. Measured at 18.9°, oxygen consumption of brain breis decreases with increasing acclimatization temperatures of the fish, apparently reaching zero for fish acclimatized at 42°, a temperature only slightly above the highest to which goldfish have been acclimatized successfully. After transfer from 27° to 12°, the rates of change in the rates of respiratory movements, oxygen consumption and brain brei oxygen absorption are about the same, 50 per cent of the changes taking place in 2 days.

It is suggested that changes in brain metabolism constitute a homeostatic mechanism which is responsible for the shift in activity and in ability to withstand lowered temperatures which accompany acclimatization to lowered temperatures.

101. AN IMPROVED METHOD FOR THE ARTIFICIAL INSEMINATION OF MICE. J. C. Kile, Jr., Biology Division, Oak Ridge National Laboratory. (Introduced by William L. Russell.) (Lantern; 15 min.)

Several methods for artificially inseminating mice were tested. The insemination of cyclic females at from 1½ to 4½ hours following mating with vasectomized males proved to be more successful than any reported method. After mid-ventral laparotomy, the sperm suspension was introduced through the wall of the uterine horn with a 28-gauge hypodermic needle. The operative procedure was performed under ether anesthesia. Of 103 inseminations made, 74 were fertile. The mean litter size was 5.97. The percentage of fertile inseminations (71.8) obtained may approach that occurring from natural matings in the inbred strain used.

102. THE RESPIRATION OF THE CECROPIA SILKWORM IN THE PRESENCE OF HIGH PRESSURES OF CARBON MONOXIDE. Howard A. Schneidermann,¹ Ned Feder, and Carroll M. Williams,² Harvard University. (Introduced by H. B. Bigelow.) (Lantern; 15 min.)

A method will be described for measuring the oxygen consumption and carbon dioxide production of small animals in the presence of positive gaseous pressures up to 10 atmospheres. The method has been used to appraise the metabolism of the Cecropia silkworm in the persence of one-fifth atmosphere of oxygen and 5 to 10 atmospheres of carbon monoxide. Results will be presented to define the role of cytochrome oxidase in the metabolism of diapausing pupae and developing adults of the Cecropia silkworm.

¹ A.E.C. Fellow in Biology.

² This study was aided by the Lalor Foundation, the DeLamar Tutorial Fund, and the American Cancer Society.

103. OXIDATIVE ENZYMES IN RELATION TO PUPAL DIAPAUSE AND ADULT DEVELOPMENT IN THE *CECROPIA* SILKWORM. Richard C. Sanborn and Carroll M. Williams,¹ University of Illinois and Harvard University. (Introduced by A. B. Dawson.) (Lantern; 15 min.)

The pupation of the *Cecropia* silkworm is accompanied by a breakdown of the distinctive cytochrome system of the larval insect. There then ensues a prolonged period of pupal diapause characterized biochemically by low titers of cytochrome *c* and cytochrome oxidase and high titers of catalase and flavoprotein. The latter, during the period of dormancy, apparently serves as a cyanide-insensitive, autoxidizable terminal oxidase. When the hormonal system of the insect functions to terminate diapause, the onset and progress of adult development are accompanied by a rapid synthesis of cytochrome *c* and by characteristic changes in the activity of cytochrome oxidase. Meanwhile, a marked decrease occurs in the titers of catalase and flavoprotein. Thus the progress of adult development is signalled biochemically by a rapid substitution of the cytochrome system for the old cyanide-stable flavoprotein metabolism of the diapausing pupa. The evidence strongly suggests that these changes are at least a part of the mechanism whereby the metamorphosis hormone terminates diapause at the cellular level.

¹ This study was aided by the Lalor Foundation and the American Cancer Society.

104. THE MOULTING-FLUID OF THE *CECROPIA* SILKWORM. Janet V. Passonneau and Carroll M. Williams,¹ Argonne National Laboratory and Harvard University. (Introduced by G. H. Parker.) (Lantern; 15 min.)

At 25°C. the adult *Cecropia* moth emerges from its pupal case 21 days after the pupa initiates adult development. At the very onset of this period, the hypodermis separates from the pupal cuticle and retracts to form an intervening space. Into the "exuvial space" thus vacated, the hypodermis simultaneously secretes a transparent, aqueous gel termed "moulting-fluid." During the first 19 days of adult development, the formation of the adult cuticle proceeds in contact with this overlying material. From the 1st to about the 11th day, the moulting-gel is without discernible action on the pupal cuticle. Midway during adult development, however, the gel is converted into a sol and begins to attack the pupal cuticle. By the 19th day the pupal endocuticle has completely disappeared leaving only the thin, crisp, sclerotized layers of the exocuticle, and epicuticle. Finally, on the 20th day, the moulting-fluid itself is completely resorbed and replaced by air.

Moulting-fluid, withdrawn from the exuvial space, is invariably a transparent, colorless, acellular, slightly alkaline material containing approximately 96% water and significant titers of sodium and potassium. The conversion of the early inactive gel into the late active sol is accompanied by a decrease in protein concentration, an increase in chitinase activity, and the first appearance of proteinase activity. Between the moulting-fluid and the underlying insect there exists a dynamic relationship. Tagged amino acids injected into the moulting-fluid are promptly absorbed and incorporated into the proteins of the adult moth.

¹ This study was aided by the Lalor Foundation and the American Cancer Society.

105. AN IMMUNOLOGICAL STUDY OF THE LARVAL-PUPAL TRANSFORMATION OF THE CECROPIA SILKWORM. William H. Telfer and Carroll M. Williams,¹ Harvard University. (Introduced by A. S. Romer.) (Lantern; 15 min.)

The blood of holometabolous insects bathes tissues which undergo extensive alterations during the course of metamorphosis. Certain tissues die, others are rejuvenated, and still others undergo rapid and progressive growth and differentiation. These events are almost certainly the end-products of a more fundamental metamorphosis at the molecular level. Attention therefore focusses on possible macro-molecular alterations that may occur in synchrony with metamorphosis—alterations which may mirror, or, indeed, be causally related to the extensive breakdown and buildup of specific tissues. We have therefore applied the Oudin immunological technique to a study of antigen changes occurring in the blood of the *Cecropia* silkworm during the larval-pupal transformation. Antisera were prepared by injecting rabbits with saline extracts of either mature larvae or pupal silkworms. Cell-free blood of similar animals was used as the corresponding antigen solutions in the tests. A maximum of six bands of precipitate was observed when either larval or pupal blood was reacted with either larval or pupal antisera, indicating that at least six different antigens are present in the blood at these stages. The same antigens were found in larval and pupal bloods. The only apparent difference between bloods at the two stages is that the antigens average one and a third times as concentrated in pupal as in larval blood. Consequently, the study has failed to reveal any qualitative changes in blood antigens during the larval-pupal transformation.

¹ This study was aided by the Lalor Foundation and the American Cancer Society.

106. IMPLICATION OF AEROBIC PHOSPHORYLATION IN THE ACTIVE CELLULAR TRANSPORT OF PHENOL RED BY ISOLATED RENAL TUBULES. R. P. Forster, Dartmouth College and J. V. Taggart, Columbia University. (Lantern; 15 min.)

Teased isolated renal tubules from fish kidneys were directly examined to observe the rate of dye transport under control conditions and after exposure to a wide variety of physical and chemical agents. Particular attention was given to a series of twelve substituted phenols. The inhibitory action of some of these compounds was associated with their capacity to interfere with the formation of energy-rich phosphate bonds in an in vitro respiring kidney enzyme system. It is concluded that the cellular transport of phenol red is at least partially dependent upon the utilization of phosphate bond energy.

107. DEPENDENCE OF CILIARY ACTION ON ACETYLCHOLINE ESTERASE ACTIVITY. Gerald R. Seaman, Department of Physiology, University of Texas—Medical Branch. (Lantern; 15 min.)

The ciliate *Tetrahymena geleii* contains an active acetylcholine esterase. The enzyme splits $0.08 \mu\text{g}$ acetylcholine/hr./mg dry weight of tissue. The enzyme does not attack butyrylcholine. At concentrations of 4×10^{-7} M, eserine and DFP inhibit hydrolysis of acetylcholine by the enzyme preparation from *T. geleii*. The in-

hibitors, at concentrations of 3.85×10^{-3} M and higher, inhibit motility of the organism. DFP and eserine apparently inhibit specifically acetylcholine esterase and not energy supplying enzymatic reactions; cells immobilized by the inhibitor treatment have the same glycolytic rate and the same rate of carbohydrate oxidation as do untreated cells. The inhibitions of motility effected by DFP and by eserine are completely reversible by washing.

DFP and eserine also inhibit ciliary activity of the frog esophagus and of the gill of *Venus mercenaria*. The inhibitions in these cases are also completely reversible by washing.

It is concluded that the fibrillar system connecting the base of individual cilium is actually conducting tissue and as such is dependent upon acetylcholine esterase activity.

108. PARALLEL ACTION OF DRUGS ON ANIMALS AND ON ELECTRICAL PHASE-BOUNDARY POTENTIALS IN VITRO. T. C. Barnes and R. Beutner, Hahnemann Medical College of Philadelphia and Medical College of Alabama, Birmingham. (Lantern; 15 min.)

Previous studies (Anat. Rec., 105: 558, '49; J. Pharmacol., 98: 2, '50) showed that the electrical action of drugs (curare, eserine) is proportional to their effects on animals (supporting the electrical theory of drug action). The present experiments suggest that strychnine acts like electroshock. One cubic centimeter containing 0.1 mg strychnine sulfate produced no electrical potential in the oil-cell containing 200 cm³ of equal parts saline and sodium oleate; 1 cm³ containing 0.1 mg strychnine from the same solution likewise had no effect when injected I.V. into a 2.8 kg rabbit. Under the same conditions 1 cm³ of solution containing 10 mg strychnine gave a negative spike of 60 mv. in the oil-cell and produced convulsions and death within 160 seconds in the rabbit. In the same way prostigmine solutions were assayed both electrically and biologically. One cubic centimeter containing 0.05 mg prostigmine gave only 3 mv. negativity in the oil-cell and no effect on a 2.6 kg rabbit. One cubic centimeter containing 0.1 mg prostigmine produced 10 mg negativity in the oil-cell and tremors and salivation in the rabbit. In morphine experiments with 20 gm mice the following data were obtained: 4 mg morphine produced no electrical effect in the oil-cell and no mortality (4 mg per 20 gm mouse). Six milligrams morphine gave 10 mv. negativity and 60% mortality while 12 mg morphine produced 15 mv. negativity and 100% mortality.

109. INDIVIDUAL SPECIFICITY IN THE FUSION OF HYDROID STOLONS AND THE RELATIONSHIP BETWEEN STOLONIC GROWTH AND COLONY FORM. Sears Crowell, Indiana University. (Lantern; 15 min.)

The stolons or hydrorhizae of colonies of *Hydractinia*, *Podocoryne*, and *Stylocystis* are extended by terminal growth and lateral branching. When a growing tip encounters another stolon of the same colony the perisarc is dissolved and the coenosarc unite. Stolons of sub-colonies derived from a single colony fuse when they meet so that a single unified colony is produced. However, stolons of sub-colonies derived from different specimens fail to fuse following contact. This specificity of

individual colonies has been observed for *Stylactis* and *Podocoryne*. It has been more thoroughly tested for male *Hydractinia* using all possible pairings of 5 colonies and 14 pairings of these and other colonies.

The fusion of stolons leads to the establishment of the net-like system of anastomizing tubes characteristic of these encrusting hydroids. The stolons of *Campanularia flexuosa* lack the ability to fuse with one another. Partly as a consequence of this the stolon pattern is racemose, in contrast to the network in encrusting species.

In *Hydractinia*, but not in *Stylactis*, the network is transformed into a solid mat, the interstices being filled by proliferation of the epidermal layers. Once a mat is established it may be extended either by proliferation of stolons or by direct growth of its edge.

110. PRELIMINARY STUDY OF THE HEMOCONCENTRATION RESPONSE OF ALBINO RATS TO ACUTE HYPOTHERMIA. A. C. Higginbotham and M. R. Siers, Florida State University. (Introduced by F. R. Hunter.) (Lantern; 7 min.)

As a part of a planned study of the physiological responses of albino rats to lowered body temperatures, the blood of control and experimental groups of rats was studied with respect to specific gravity, volumes of cells and plasma, and hemoglobin content. Control animals were etherized and their body temperature was maintained relatively normal during the one-hour observation period. Experimental animals were etherized and cooled by submersion in an ice-water bath for a similar length of time.

Hemodilution occurred in the etherized control animals, differing in this respect from dogs in which hemoconcentration occurs under similar conditions.

Lowering the body temperature of the experimental animals approximately 10–15°C. resulting in hemoconcentration. Further depression of their body temperature brought about hemodilution.

Embryology

Section B

111. EARLY EMBRYOLOGY OF THE DOG. H. T. Gier, Kansas State College, Manhattan. (Lantern; 8 min.)

Dogs were bred under controlled conditions. Laparotomies were performed at intervals ranging from one day after copulation through full term, and embryos were recovered by flushing the uterus or by fixation and sectioning.

Ovulation occurs at approximately the middle of the estrus period and fertilization is delayed several days pending completion of maturation. The first cleavage division is complete at six or seven days after ovulation, and the second division within 24 hours thereafter. The embryos reach the uterus in the 2- or 4-cell stage, and development proceeds slowly with the embryo floating free within the uterine cavity until the eighteenth day. The blastocyst stage is reached on the tenth day. The zona pellucida is lost on the fifteenth day when the blastocyst is approximately

1.5 mm in diameter and the endoderm is beginning to differentiate. The endodermal sac is complete on the sixteenth day, and the neural folds are distinct. The first somites appear on the eighteenth day; head fold and amniotic folds on the nineteenth or twentieth day. The amnion is completed on the twenty-first day, and placentation is underway.

112. PLACENTATION IN THE DOG. D. Romanucci, Kansas State College, Manhattan. (Introduced by H. T. Gier.) (Lantern; 7 min.)

During the first 18 days after ovulation, the egg and resulting embryo float free within the cavity of the oviduct and uterus. The chorionic villi begin to form at about 18 days as pseudopodia-like extensions from the cells of the trophoctoderm which grow into the openings of the uterine glands. Secondary branching of the original villi results in the formation of other chorionic villi. Following formation of the secondary villi, digestion of the mucosa begins concurrently with penetration of the villi by allantoic blood vessels, about 20 days after ovulation. Digestion continues rapidly during the next ten days, resulting in extensive destruction of the compact layer.

Definite swelling of the uterus is visible at 18 days after ovulation when the blastocyst is 3×5 mm and the embryo is beginning to show somite formation. At this time, the beginnings of chorionic villi are visible. In the next three days, the chorionic vesicle nearly doubles in linear measurements, chorionic villi grow into the uterine glands and digestion of the mucosa begins. By the twenty-fifth day, the chorionic villi are completely established within the endometrium.

113. SIMULTANEOUS ANTERIOR AND POSTERIOR REGENERATION AND OTHER GROWTH PHENOMENA IN CLYMENELLA AND RELATED POLYCHAETES. Gairdner B. Moment, Goucher College and Duke University Marine Laboratory, Beaufort, N. C.

Isolated pieces 10 and 13 segments long cut from five different levels of *Clymenella torquata* regenerate the correct number of segments to restore the 22 characteristics of the species. The number of segments regenerated anteriorly increases in exact proportion as the origin of the piece becomes more posterior, while the number of segments regenerated posteriorly decreases correspondingly. Thus isolated pieces maintain their origin with respect to the worm as a whole. Because segment 6 always regenerates anteriorly segment 5 with its characteristic specializations regardless of whether segment 6 is followed by 10 or by 13 segments or by the intact hind end of the worm, Child's theory that the nature of the regeneration depends on some kind of a ratio between the cut surface and the remainder of the worm would seem to fall to the ground.

In posterior regeneration from segment 15, in *C. torquata* and in *Axiiothella mucosa* all new segments appear simultaneously and very small. In *A. rubracincta* they appear seriatim with the smallest at the tip as in earthworms (*Eisenia foetida*). In *C. torquata* adults however, the length though not the width, of each segment decreases from segment 15 to the end of the worm.

114. THE ACCUMULATION OF PHOSPHATASE IN THE DUODENUM OF MOUSE EMBRYOS AND YOUNG MICE. Florence Moog, Washington University. (Lantern; 12 min.)

Previous studies have shown that in the chick embryo alkaline phosphatase in the duodenum increases about 100-fold between 18 days of incubation and the day after hatching. The increase is coincident with the appearance of the enzyme in the striated border. In the mouse the enzyme undergoes a 10-fold rise between the 17th day of gestation and the day of birth, and then declines slightly. At the beginning of the third week of post-natal life the enzyme rises sharply again, and within 48 hours arrives at a level about 15 times above that of the nursing period. This change is independent of the growth rate or weight of the individual. In both periods the accumulation is localized in the striated border. The double onset of functional activity (intake of milk and intake of solid food) in the mouse is thus correlated with two periods in the development of a digestive enzyme.

In the 4th post-natal week the phosphatase concentration falls somewhat, and this fall is hastened by putting the animals on a diet of solid food only. If the animals are deprived of food at the end of the second week, the increase of phosphatase still occurs; but at the end of the accumulation period, starvation results in a decline. In older mice starvation has little effect on phosphatase concentration.

115. EFFECTS OF ETHYL CARBAMATE (URETHANE) ON THE EARLY DEVELOPMENT OF THE TELEOST, *BRACHYDANIO RERIO*.¹ Kenneth Hisaoka, University of Western Ontario, London, Canada. (Introduced by Helen I. Battle) (Lantern; 15 min.)

Since ethyl carbamate possesses certain specific carcinogenic properties and has also been used in the treatment of leukemia, the desirability of demonstrating its effects on the differentiation of embryonic structures is indicated. Zebra fish (*Brachydanio rerio*) were subjected, during stages from early cleavage to the initial appearance of optic pigmentation, to graded concentrations of urethane from 0.05 to 1.25 percent, for different periods. Development was progressively retarded with increasing concentrations from 0.25 percent and almost completely inhibited at 1.25 percent, while hatching failed in concentrations of 0.5 percent and over. Developmental anomalies of various types and degrees were produced from .25 to 1.25 percent, and were dependent upon concentration, duration of exposure, and to some extent on the initial stage of development. These included oedema of the coelom, commencing in the pericardial cavity with coincident absence of the typical flexure of the cardiac tube, and progressing posteriorly at high concentrations to include the whole peritoneal cavity; spasmodic or complete absence of heartbeat; incomplete circulation accompanied by stagnation in sinuses of the extra embryonic area; contortion and reduction of the body axis involving the notochord and myotomes of the tail and trunk; various degrees of cyclopia; incomplete and dilated brains. A heavy irregular cellular aggregation appeared frequently on the anterior surface of the pericardial cavity. The more advanced the developmental stage attained prior to exposure, the greater the resistance to the chemical. The anomalies induced are constant and repro-

ducible and correspond in general to types reported by various authors employing a variety of other agents and cannot be considered as specific for urethane.

¹ This investigation was supported by a grant from the National Cancer Institute of Canada.

116. THE MODIFICATION OF DEVELOPMENTAL PATTERN IN THE SAND DOLLAR WITH SODIUM SELENITE. Olin Rulon, Northwestern University. (Lantern; 15 min.)

Newly fertilized eggs, 6-, 12-, and 24-hour larvae of *Dendraster excentricus* were exposed to sea water solutions of sodium selenite (M/200-M/3200) for periods ranging from 6 to 72 hours.

The most effective treatment (M/400-M/800 for 18-24 hours and beginning with the newly fertilized egg or 6-hour blastula) retarded slightly the rate of development. On reaching the gastrula stage most of the larvae failed to develop ventrodorsally. Instead, the gastrula tended to elongate in the polar direction and to develop a constriction or neck at approximately $\frac{1}{3}$ ths the distance from the apical to basal end. This resulted in the development of a large basal lobe and somewhat smaller apical lobe. Usually a large ciliated tuft appeared at the tip of the apical lobe while ciliated bands developed around the neck and bottom of the basal lobe. The entoderm usually invaginated and differentiated into a 3-segment gut although under certain conditions whole or partial evagination occurred. When the stomadaeum formed it was in the basal lobe. There was an excess of cells (mesenchyme?) given off into the interior of the larva which differentiated into muscle but not skeletal elements. With other ranges and exposure periods forms appeared which graded toward the normal pluteus or toward complete inhibition.

The partial or complete loss (or inhibition) of certain correlative factors associated with the polar and ventrodorsal gradient systems of this egg is given in interpretation.

117. EFFECTS OF THIOUREA, THIOURACIL, PHENYLTHIOUREA, AND PROPYL-THIOURACIL ON MORTALITY, GROWTH, AND DIFFERENTIATION OF *DROSOPHILA MELANOGASTER*.¹ Morris H. Harnly and E. D. Goldsmith, Washington Square College of Arts and Science and Department of Histology, College of Dentistry, New York University. (Lantern; 15 min.)

The mortality curves of *D. melanogaster* fed thiourea in concentrations of 5-15 mg percent (to complete lethality) show that Oregon wild is the least susceptible, Woodbury wild and black have superimposable curves and are more susceptible; ebony¹¹ is the most susceptible. Phenylthiourea fed in concentrations of 15-120 mg percent (to complete lethality) proved much less toxic. The black and ebony¹¹ mortality curves were superimposable and showed the highest resistance; Woodbury wild was more susceptible; and yellow was very sensitive to this compound. The Woodbury wild viability range on thiouracil was the same as on thiourea but the mortality values were significantly higher for thiouracil at all points within this range. Preliminary tests on propyl-thiouracil indicate it resembles more nearly phenylthiourea in toxicity. It is evident that the re-

sistance to these compounds varies with the genotype, and the sequence of genotype susceptibility varies from drug to drug.

Thiouracil, the most toxic of these compounds, has no effect on either the rate of growth or differentiation. Thiourea not only causes abnormal types of metamorphosis in some cases, but also retards equally the rates of growth and differentiation. Phenylthiourea is the least toxic of these three but retards markedly and unequally the rates of growth and differentiation. The mutant black is retarded $2 \pm$ days in development to the imago; ebony¹ $3 \pm$ days; and Woodbury wild $5 \pm$ days. Growth is not retarded proportionally. The adult imagos are half to two-thirds the size of their sib controls.

¹ Aided by a grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

118. THE GROWTH OF MESENCEPHALIC V NUCLEUS CELLS AS A METAMORPHIC EVENT IN ANURANS. Jerry J. Kollros,¹ Virginia Pepernik, Robert Hill, and Jane C. Kaltenbach, The State University of Iowa. (Lantern; 15 min.)

Cells of the mesencephalic nucleus of the trigeminal nerve can be identified in small anuran tadpoles. In *Rana pipiens* these cells grow slowly until stage XIV (late mid-larval period) and more rapidly until the end of metamorphosis. At that time the average cell has two-thirds the volume of the average adult cell, although nuclear size is virtually that of the adult.

Dependence of cell growth upon thyroid hormone was demonstrated (a) through stimulation of the whole animal in thyroxin solutions containing 1 part of thyroxin to 20-40 million parts of water, and (b) through local stimulation with implants of thyroxin-cholesterol pellets into the cranial cavity just anterior to the midbrain. In the first group the mesencephalic V nucleus cells grew to postmetamorphic or adult size before any other metamorphic event had gone to completion. In the second group local stimulation of cell growth was noted; cells near the implant were larger than were cells farther away, resulting in lateral asymmetries in the sizes of mesencephalic V nucleus cells. Regions distant from the implant (jaw, eye, leg) showed only mild metamorphic stimulation. These studies reveal (a) the dependence of the mesencephalic V nucleus cells for their growth upon the level of thyroid hormone in the body fluids, and (b) their independence, so far as growth is concerned, of metamorphic changes in the peripheral structures (presumably jaw muscles) to which the cells send sensory fibers.

¹ This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

119. DIFFERENTIATION BY LOSS OF BIOCHEMICAL SYNTHETIC ABILITY. Clement L. Markert, University of Michigan. (Lantern; 15 min.)

During the embryogeny of higher organisms cells become differentiated from one another in part at least through the development of distinctive relative concentrations of intracellular enzymes. The mechanism by which the various patterns of enzyme concentrations are achieved is still unknown. However, similar kinds of enzymatic and correlated morphological changes are induced in the fungus

Glomerella by single genetic changes—thus suggesting the feasibility of a genetic-type mechanism for differentiation. On a simple chemically defined medium, the hyphal cells of this fungus grow in a characteristic pattern which imparts a distinct morphology to the entire mycelium. By single gene changes the biochemical synthetic ability of these cells can be reduced and their enzymatic content changed. With each loss in synthetic ability the cells frequently undergo changes which grossly alter the morphology of the mycelium. Furthermore, the genetic loss of a simple synthetic capacity, e.g., the ability to synthesize a particular amino acid, frequently results in great enhancement of some other synthetic ability such as the production of tyrosinase. Thus, genetic inactivation of enzyme synthesis in these fungus cells produces changes similar to those observed in the differentiating tissues of higher organisms.

120. CARBOHYDRATE METABOLISM IN INSULIN-TREATED CHICK EMBRYOS. Edgar Zwilling, University of Connecticut. (Lantern; 10 min.)

Free carbohydrate, glycogen and total carbohydrate have been assayed in insulin-treated (5 units injected into the yolk-sac at 5 days) and control embryos and yolk-sac membranes at 6, 8, 10 and 12 days. Bound carbohydrate was calculated from these data. The object of this study was to discover how the insulin produced the hypoglycemia which has been reported earlier and, if possible, gain some further information about the morphological effects (micromelia) of such treatment.

It was found that the glycogen content and total carbohydrate of the yolk-sac membranes increased after insulin treatment and that the other carbohydrates were diminished. All carbohydrate fractions decreased in the embryo proper. Free carbohydrate in the fluid yolk was not altered by insulin.

These data indicate that insulin (applied as above) acts directly on the yolk-sac membrane and that the alterations in the carbohydrates of the embryo result from the decreased amount of sugars released from the membrane. It is likely that these effects are the initial steps in the production of the morphological derangements in the embryo.

121. ECTRODACTYLISM (SPLIT HAND) INHERITED AS A DOMINANT WITH LOW PENETRANCE. Charles D. Howell, Muskingum College. (Lantern; 15 min.)

The trait was found in 15 individuals who were all traced to one common ancestor. The possibility of a recessive factor being involved is considered. In spite of the fact that the 15 affected individuals have a total of 44 normal sibs, it seems impossible that the descendants of this one individual would have had so great an opportunity to carry the gene, and also to marry carriers outside the family. Various possibilities are considered. The postulation of a dominant with low penetrance fits the occurrence of the trait in families of cousin marriages as well as in ten marriages of non-cousins.

General Morphology and Ecology

Section C

122. THE LOCAL EFFECT OF VITAMIN A ON RAT EPIDERMIS. Joseph Sabella, Howard A. Bern and Raymond H. Kahn, University of California, Berkeley. (Lantern; 7 min.)

In view of the relation between hyperkeratosis and avitaminosis-A and of recent observations indicating the ability of locally applied vitamin A to counteract partially the vaginal keratinization in rats resulting from estradiol treatment, histologic sections of rat skin were studied to determine whether or not topical applications of vitamin A affected normal epidermal keratinization. Shaved patches of skin on the dorsum of female rats, ovariectomized two to three weeks previously, were exposed continuously to sesame oil solutions of vitamin A alcohol, estradiol benzoate, or estradiol benzoate with vitamin A. The oil solutions were applied twice daily for 10 days by injection into a gauze pad held in place over the shaved area by means of an adhesive bandage. Measurements of the thickness of the total epidermis and of the several layers comprising it were made on sectioned material.

No significant alteration in epidermal thickness over values for the untreated castrated females was observed in animals treated with sesame oil alone or with estrogen in sesame oil. A significant increase in thickness was noted in both the vitamin A-treated and the estrogen-vitamin A-treated rats, due largely to the increased extent of the stratum granulosum. In oil-treated and estrogen-treated animals, the stratum granulosum was denser than that of the untreated rats; however, in the vitamin A-treated groups, keratohyalin granules were distributed more extensively throughout the superficial layers of the epidermis than in those animals not receiving the vitamin. Evidence for a decreased rate of keratinization due to vitamin A will be discussed.

123. METABOLIC TYPES AND GROWTH TYPES. Ludwig von Bertalanffy, Department of Biology, Faculty of Medicine, University of Ottawa. (Introduced by Daniel P. Quiring.) (Lantern; 15 min.)

The problem of the dependence of metabolism on body size is usually discussed in terms of the surface rule which states that basal metabolism is proportional to a surface of $2/3$ power of weight. The surface rule, as stated by Rubner, was explained by the exigencies of homeothermy. Even in recent discussions, homeothermic animals are almost solely taken into consideration. When the problem is considered on the basis of comparative physiology, investigation shows (1) that the rule holds for poikilothermic vertebrates and certain invertebrates and has a wide application, but that current explanations are too restricted; (2) that there are many groups for which the surface rule does not hold. The work of the author and his group has led to the statement of metabolic types in respect to the relation between metabolism and body size. Three such types have been distinguished, namely: metabolism is proportional (1) to a $2/3$ power of weight (vertebrates, especially fish, lamellibranchs, isopod crustaceans, etc.); (2)

to weight of itself (insect larvae, *Orthoptera*, annelids, *Helicidae*); (3) is intermediary between surface and weight proportionality (*Planorbidae*).

A strict connection between these metabolic types and types of animal growth has been established. In the first and third types, growth approaches a steady state, with characteristic differences in the growth curves; in the second, growth is exponential, does not attain a steady state, and is intercepted only by metamorphosis or seasonal cycles. These types are in agreement with, and can be deduced from, the theory of growth advanced by the author.

124. VASCULAR, NEURAL AND SKELETAL ANOMALIES ASSOCIATED WITH THE SHORT EAR GENE IN THE MOUSE. Margaret C. Green, Ohio State University. (Introduced by C. E. Venard.) (Lantern; 10 min.)

The short ear gene reduces the size of many bones and cartilages and causes waviness of the tail and a low incidence of hydronephrosis. A search was made for a causal relationship between the various effects based on the hypothesis that the wavy tail and hydronephrosis are neuromuscular in origin and result from neural abnormalities secondary to the cartilage defects. This led to the discovery of abnormalities of the vertebrae, blood vessels, and autonomic nerves. In short ear mice the ventral walls of the vertebralarterial canals or transverse foramina of the cervical vertebrae are often absent. The anapophyses of the posterior thoracic and anterior lumbar vertebrae are small or absent. The transverse processes of the lumbar vertebrae and the chevron bones of the tail are smaller than normal. The right renal artery, which normally passes dorsal to the posterior vena cava, usually passes ventral to it in short ear mice. Occasionally the abdominal aorta is displaced ventrally to pass ventral to the left renal vein. There is a tendency in short ear mice for the greater splanchnic nerve which occurs in normal mice only as a short connection between the first lumbar ganglion and the coeliac ganglion to lead directly from the last thoracic ganglion to the coeliac ganglion. The relation of these differences to wavy tail and hydronephrosis is not yet known.

125. THE GILL AREA OF MARINE FISHES — A COMPARATIVE STUDY. I. E. Gray, Duke University. (Lantern; 15 min.)

A study of twenty-seven species representing seventeen families of marine fishes reveals that the area of gill surface is correlated with the habits of the species. Pelagic fishes like the herrings, mackerels and jacks have large areas of gill surface compared to bottom inhabiting fishes such as flounders, eels, toadfish and goosefish. The correlation is with the amount of energy expended by the fish rather than with the amount of oxygen available in the habitat.

126. A THRESHOLD CONCEPT FOR INTERPRETING VARIATIONS IN THE AXIAL SKELETON OF INBRED STRAINS OF MICE. E. L. Green, Ohio State University. (Lantern; 15 min.)

Several highly inbred strains of house mice (C57blk, dba, C3H, BalbC, P, NB, Sese-C1, Sese-C2, Sese-Ab) exhibit variations in the number of thoracic and

lumbar vertebrae both within and between strains. Some strains (C57blk, NB) contain chiefly mice with 13 thoracic and 6 lumbar vertebrae, others (dba, C3H) contain chiefly the 13/5 type, one (P) contains chiefly the 12/6 type, one (Sese-C2) contains a large number of the 13/7 type, and one (BalbC) contains a variety of types including 13/6, 14/5, 13/7, 14/6. This diversity of skeletal types may be brought into a unified conception by supposing the existence of a scale on which combinations of genetic and non-genetic factors may be measured. Each elementary factor, whether genetic or non-genetic, may be supposed to have "plus" and "minus" alternatives. The scale location of a given combination of factors measures the density of "plus" factors. Since vertebrae occur only in whole numbers, many different factor combinations may produce the same number of vertebrae. Only when the density of "plus" factors exceeds a critical amount or threshold is a greater number of vertebrae produced. On this view, at least two thresholds must be assumed in order to distinguish between 25, 26 and 27 presacral vertebrae, and another two to distinguish between 12, 13 and 14 thoracic vertebrae. Tests of the threshold scale have shown that it satisfies certain criteria of adequacy for interpreting the results of matings between the pure strains of mice.

127. STUDIES ON NUTRITIONAL ANEMIA IN *TRITURUS VIRIDESCENS*. Sophie Jakowska and Ross F. Nigrelli College of Mt. St. Vincent and New York Zoological Society. (Lantern; 15 min.)

The nutritional anemia in the newt is characterized by the formation of numerous pseudoerythroplastids (Nigrelli, 1929; Jordan, 1938). These are basophilic red blood cells resulting from a gradual enlargement of the nucleus and the development of a fine chromatin network without a breakdown of the nuclear membrane; the nucleus eventually occupies almost the entire cell and very little cytoplasm remains visible.

The pseudoerythroplastids stain greenish with Wright's instead of reddish and deep blue, the typical appearance of the cytoplasm and nucleus, respectively, of the normal erythrocytes. With Feulgen and methyl green, it is apparent that the desoxyribosenucleic acid content does not change during the development of these abnormal cells. A diffuse reaction results in the enlarged nuclei of the pseudoerythroplastids with Sudan Black B, which in the normal erythrocytes of newts demonstrates only sudanophilic perinuclear bodies.

The anemia appeared in newts after one month in captivity and when kept on a limited diet of *Tubifex*. The majority of salamanders became emaciated, eventually taking little or no food. The internal organs were reduced in volume and the spleen was thin, fibrous and haemopoietically inactive. No deaths, however, occurred among these animals. Other newts with the anemia were robust and active in feeding and behavior. The internal organs were considerably increased in volume; the enlarged spleen was almost exclusively filled with pseudoerythroplastids in late stages of development. Similar cells with narrow rims of cytoplasm containing haemoglobin were also present in the liver. Deaths in this group occurred frequently.

128. STRUCTURE OF THE VERTEBRATE LIVER. Hans Elias, The Chicago Medical School. (Lantern; 15 min.)

Previous workers have described the liver of the non-mammalian vertebrates as a net-like, tubular end.

The livers of the following forms were examined: *Myxine*, *Petromyzon*, *Squalus*, *Gymnothorax*, *Cyprinus*, *Carassius*, *Amia*, *Necturus*, *Hyla*, *Rana*, *Eumeces*, *Alligator*, *Clemmys*, *Emys*, *Terrapene*, *Gallus*, *Sturnella*, *Ornithorhynchus*, *Echidna*, *Didelphis*, *Lepus*, *Equus*, *Capra*, *Bos*, *Canis*, *Felis*, *Homo*.

The application of stereographic and statistical methods to the problem of liver structure shows that this organ is not a tubular gland. The liver of members of all classes and of many orders of vertebrates (with the exception of that of the *Petromyzonidae* during metamorphosis and in adulthood) is a continuous mass of cells, tunneled by more or less cylindrical, anastomosing lacunae which contain the sinusoids. The walls between neighboring sinusoids are predominantly two cells thick, but one cell thick walls occur locally in all specimens.

The liver of the mammals (*Monotremata*, *Marsupialia* and *Placentalia*) differs from that of the lower vertebrates in the thickness of the walls. The mammalian liver plates are uniformly one cell thick.

In lower vertebrates, the bile canaliculi run between both cell layers. Closed meshes of bile canaliculi are not very frequent. In the mammals, the tough walled bile canaliculi form continuous nets. Meshes embrace individual cells contributing to the mechanical stability of the liver tissue.

Aside from these differences, the structure of the liver is the same in all vertebrate classes.

129. THE RED AND BLACK PIGMENTS OF *PLETHODON CINEREUS*. Emanuel C. Hertzler, Kent State University. (Introduced by Frederick H. Test.) (Lantern; 10 min.)

Both red and black pigments of the salamander, *Plethodon cinereus*, occur as discrete granules in dendritic cells which surround the mucus and poison glands. Neither pigment is soluble in water or organic solvents. The black pigment is dissolved out of whole skin by exposure to 2% KOH for 24 hours at room temperature. Spectrophotometric examination of the resulting extract shows strong absorption in the ultraviolet end of the spectrum with gradually increasing transmission through the visible and infrared spectra. The red pigment is extracted by 2% KOH in 10 minutes at room temperature. Its spectral absorption is also greatest in the ultraviolet but it is characterized by a very strong absorption band at 345 m μ . Measurements of the spectral reflectance of living skin have been made with an adaptor devised for use with a Beckman spectrophotometer. These reflectance measurements are correlated with the transmission characteristics of the pigment extracts.

130. **ROLE OF FOOD HABITS IN INTERSPECIFIC COMPETITION BETWEEN MICHIGAN GARTER SNAKES.** Charles C. Carpenter, University of Michigan. (Introduced by F. H. Test.) (Lantern; 15 min.)

In a study of the ecology of Michigan garter snakes for three seasons (1948-50) on 50 acres in Washtenaw County, Michigan, 425 food records were obtained by forced regurgitation from 592 Common Garter Snakes (*Thamnophis sirtalis*), 362 Ribbon Snakes (*Thamnophis sauritus*), and 227 Butler's Garter Snakes (*Thamnophis butleri*) living closely associated on this area of varied habitats. The numbers of food records obtained for each species corresponds to its abundance.

Except for a few leeches, earthworms comprise all food records for Butler's Garter Snake. The Ribbon Snake is almost entirely an amphibian feeder, a few small fish and caterpillars being the only other items recorded. The Common Garter Snake feeds on a variety of vertebrates and invertebrates in the proper size range, earthworms being most common.

Habitat data on these three species correlate well with the food preferences indicated. One of the reasons that these three species of garter snakes are able to exist in large numbers on the same area is their differing food preferences.

Adult Common Garter Snakes show a much greater preference for amphibians than do juveniles. Adult Ribbon Snakes feed on larger species of amphibians than do the young. Seasonal variations in food are related to availability of prey species.

131. **RESPONSE OF A POPULATION OF WILD HOUSE MICE (*MUS MUSCULUS*) TO RESTRICTED FOOD SUPPLY.** Robert L. Strecker and John T. Emlen, Jr., University of Wisconsin. (Lantern; 10 min.)

A population of wild house mice was established by releasing five pairs of mice in a large room well supplied with cover, but containing no mice previously. An excess of water and a measured amount of food were supplied at several locations in the room. The amount of food consumed was measured each day, and the population growth estimated in these terms. As long as there was an abundance of food available, there was very little egress from the room. As the population increased toward the point where all of the food was being consumed each day, there was a great increase in the amount of egress from the room. Both sexes took part about equally in the movement out, with all ages of mice except the very young represented. The rate of reproduction in those remaining behind in the room was maintained at a normal level. This is in sharp contrast to a comparable population studied concurrently, in which there was no means of egress available. In this latter population as the total food consumption was reached there was a complete cessation of reproduction.

132. **AERIAL OBSERVATIONS OF HOMING PIGEONS.** Harold B. Hitchcock, Middlebury College. (Lantern; 12 min.)

Observations from light airplanes were made of flights of youngsters, one- and two-year-old racing pigeons homing 9-107 miles to Middlebury, Vermont, and of youngsters homing 85-187 miles to Fort Monmouth, New Jersey.

Birds usually fly 10-20 minutes in area of release before leaving. After orientation flight a fairly straight course is usually followed for some time, even if not toward loft. Initial direction of flight may be affected by mountains or large bodies of water, but after a direction has been chosen geographical and cultural features exert little influence. Usual altitude of flocks on a good course or in home territory is slightly above treetops, or lower in open country. When confused they may ascend 1000 feet above ground. Narrow valleys are crossed at approximate height of their walls. Elevations in path of flight may be anticipated by climbing before actually encountering them. While homing the flock formation is that of a slightly convex line, birds side by side, close together, with considerable changing of position by individuals. Confusion is displayed by loose formation and disintegration of flock; birds sometimes land when confused. Hawk attacks may scatter flock, the birds increasing speed, some climbing high and others hiding in trees. Other pigeons met in flight seem not to influence flock unless birds are already confused.

133. A FACTORIAL STUDY IN THE SYSTEMATICS OF *KALOTERMES*. Clyde P. Stroud, University of Chicago. (Introduced by A. E. Emerson.)

Multiple factor analysis is a mathematical technique suitable for the exploratory investigation of the phylogenetic interrelations of systematic categories. A factorial analysis of general morphological patterns in 50 species of termites of the genus *Kaloterme*s demonstrated five general factors for the soldier caste and six factors for the imagos. These factors account for the observed correlations among 14 measured characters in each caste. Some of the factors can be interpreted in terms of the phylogeny of the genus.

PAPERS PRESENTED BY TITLE

Cellular Physiology

134. A PHASE CONTRAST MICROSCOPY STUDY OF X-RAY-INDUCED PYCNOSIS IN THE LIVING CELL. Theodore N. Tahmisian and Dorothy M. Adamson, Argonne National Laboratory.

The effect of x-radiation ranging from 25,000 r to 200,000 r can be kept latent in *Melanoplus differentialis* embryos for six months by maintaining them at sub-metabolic temperatures. If diapause eggs are irradiated and left at 25°C., pycnosis of the nuclei takes place by eight days. When the eggs are irradiated and placed immediately at 0°C. and kept at this temperature for six months no morphological or physiological changes are observed upon returning them to 25°C. However, pycnosis occurs by eight days at 25°C. depending not only on the irradiation dosage but also on the metabolic activity of the cell. Pycnosis in the living cell as observed by phase contrast microscopy resembles fixed material morphologically. Physiologically there is a difference in the physical state of the nuclei when observed in the living condition. Most nuclei show a drop in viscosity at the time of pycnosis as judged by the increase in Brownian movement. Vital staining

with Janus green indicates that the mitochondria usually clump as a separate body whereas, the chromatin material becomes pycnotic containing lipid impregnated bodies.

135. CHANGES IN THE NUCLEUS OF AMOEBIA PROTEUS FOLLOWING REMOVAL FROM THE ORGANISM.¹ Robert Kassel and M. J. Kopac, Department of Biology, Washington Square College of Arts and Science, New York University.

The culture medium was drained after the amebas became attached to a coverslip and replaced by several changes of the test medium. The nuclei were removed micrurgically from the ameba and directly transferred to the test medium. The isolated nuclei, kept under continuous observation at $450\times$ magnification, were simultaneously fixed and stained with aceto-orcein.

Isolated nuclei resembled normal nuclei, *in situ*, not only in size and shape but also in the distribution of peripheral granules when transferred either to (1) medium A (KCl 0.042 M + NaCl 0.017 M + CaCl_2 0.0015 M + MnCl_2 0.001 M), (2) medium B (KCl 0.042 M + NaCl 0.017 M), (3) B + ethylene-diamine tetracetic acid (10–20 mg/cm³), or to (4) B + protamine (0.5–1.0 mg/cm³).

All peripheral and other granules stainable by orcein disappeared when the nuclei were transferred to B + hexametaphosphate (10 mg/cm³). When transferred either to distilled water or to B + disodium phosphate (20 mg/cm³), the isolated nuclei first became hyaline and later the granules reappeared.

The nuclear membranes slowly disintegrated with extrusion of the peripheral granules when the nuclei were transferred to media rich in calcium (0.003 + M) or to B + desoxyribonucleate (1 mg/cm³).

These observations indicate the value of micrurgical studies in evaluating media which may be used in homogenizing cells for the isolation and fractionation of subcellular particulates. The continuous observation, especially of the larger cytoplasmic inclusions, enables one to detect early and subsequent changes in the morphology of the isolated particulates.

¹ These investigations were supported by Grant C-843, National Cancer Institute, U.S. Public Health Service.

136. ANAEROBIC PICKUP OF P^{32} BY SEMINAL BULL SPERM. David W. Bishop, University of Massachusetts.

The rapid uptake of radioactive phosphorus by seminal bull sperm has previously been demonstrated (Anat. Rec., 101: 81, 1948). Maximum pickup occurs within one hour in sperm resuspended in Tyrode's solution (37°C.) and exposed to P^{32} as inorganic phosphate. Biochemical fractionation of washed sperm after exposure to P^{32} demonstrates 80–90% of the phosphorus which enters the cells to be in the TCA-soluble fraction, containing hexose phosphate esters. Anaerobic conditions (commercial nitrogen), while depressing oxygen consumption, increase phosphorus pickup over the aerobic value by 20–50% at one hour. Likewise excess glucose favors glycolysis and increases P^{32} uptake over the control value. No consistent P^{32} turnover was demonstrated in lipid or protein fractions during these experiments (two hours) under either aerobic or anaerobic conditions.

137. MOTILITY OF HEADLESS SPERM TAILS. David W. Bishop, University of Massachusetts.

Mammalian spermatozoa are mechanically fractionated through sonic vibration (or cavitation) developed by a nickel magneto-constriction transducer operated at 8900-9000 c.p.s. and 50 watts. Separation of heads from middle pieces and tails occurs within 20 minutes in 100% of the cells in bull ejaculates and rabbit tubal samples. At 10 minutes of vibration most of the cells are fractionated, but some intact cells remain active. After the loss of the head, the remainder of the sperm including the middle piece and tail portion proper may continue to display motility. Though considerably slowed down, these headless sperm have three motility components: rapid lateral vibratile motion, slow flexion, and occasionally slow forward movement. Motion has been observed in the sperm tails suspended in Tyrode's solution for as long as one hour following fractionation. Seminal sperm are less satisfactory than tubal sperm for these observations owing to microbiological contamination of the semen at the time of ejaculation. The persistence, characteristics, and possible significance of this motility is subject to further investigation.

138. TEMPERATURE-PRESSURE EFFECTS ON THE RATE OF BEAT OF TADPOLE HEART FRAGMENTS IN TISSUE CULTURE. Joseph Landau and Douglas Marsland,¹ Washington Square College of Arts and Science, New York University.

Temperature proves to be critical in determining both the sign and the magnitude of the pressure effect on the cardiac rate. At temperatures below 12°C., i.e. in the region where the log.rate/temperature curve does not show any deflection from linearity, the immediate effect of pressure (4000 lbs./in.²) is to slow the beat, whereas at temperatures above 15°C., this pressure greatly accelerates the rate. In the intermediate range (12°-15°), the heart rate displays very little pressure sensitivity.

The simplest interpretation of these results is based on the assumption that the main rate controlling reaction in cardiac tissue, possesses a positive ΔV and is suppressed by pressure. At higher temperatures, a reversible thermal deactivation of the enzyme system catalyzing the rate controlling reaction becomes significant, so that pressure accelerates the beat by reactivating the enzyme system. At intermediate temperatures, on the other hand, these two effects tend to cancel one another.

¹ Aided by grant C807c from the Cancer Research Grants Branch of the National Institute of Health, U. S. Public Health Service.

139. CELL DIVISION: A TEMPERATURE-PRESSURE STUDY OF THE CLEAVAGE POTENTIAL IN THE EGGS OF THE SAND DOLLAR (*ECHINARACHNIUS PARMA*). Douglas Marsland¹ and Joseph Landau, Washington Square College of Arts and Science, New York University; and the Marine Biological Laboratory.

As in the case of the Sea Urchin egg, temperature plays a critical role in determining the magnitude of pressure required to halt the cleavage furrows in the eggs of the Sand Dollar. However, the Sand Dollar, being a more northerly species, possesses eggs which can cleave at lower temperature (5°C.); and at each tem-

perature, higher (by about 1000 lbs./in.²) pressures are required to stop cleavage.

These observations give further support to the gel contraction theory of cytokinesis and provide a clue to the problem of how the intracellular gel systems of deep sea animals are adapted for coping with extreme conditions of low temperature and high pressure.

¹ Aided by grant C807c from the Cancer Research Grants Branch of the National Institute of Health, U. S. Public Health Service.

140. EFFECTS OF SPECIFIC NUTRIENTS ON POPULATION GROWTH OF *TETRAHYMENA GELEII* W. Sister M. Stanislaus Huddleston, I.H.M., Marygrove College, Detroit, Michigan.

The ciliate, *Tetrahymena geleii* W, was grown in a standard proteose peptone medium to which were added, singly and in combination, the nutrients of the Kidder-Dewey completely synthetic medium (Kidder, G. W. and Dewey, V. C., *Arch. Biochem.*, 20: 433-443. The data indicate that exhaustion of known single nutrients is not the cause of cessation of growth, although dl-isoleucine, and riboflavine augmented population growth somewhat and dl-phenylalanine, dl-valine, pteroylglutamic and nucleic acids inhibited growth somewhat. The pattern of the population curve is attributable to combinations of nutrients. The data also emphasize the importance of concentration of available food and suggest (a) that the life span of this protozoan varies inversely as the concentration of food, (b) that, in terms of population longevity and total cell material, lower concentrations of proteose peptone appear to be better media for this ciliate than are higher concentrations.

141. THE EFFECT OF PYRIBENZAMINE ON THE MOVEMENT OF HISTIOCYTES AND LEUCOCYTES IN TADPOLES.¹ Lawrence E. Feightner, Alice Berman Roth and S. Meryl Rose, University of Illinois.

Jancso (*Nature*, 160, 1947) has shown that cells of the reticuloendothelial system are not actively phagocytic in the presence of antihistamine. We have attempted to learn a bit more concerning the effect of an antihistamine on histiocytes and leucocytes.

Twelve to 20 mm *Rana pipiens* tadpoles anesthetized in 1:12,000 MS222 (Sandoz) have been examined at 430 $\times$ and 970 $\times$ magnification in Clark chambers.

Forty-five histiocytes in 11 tails have been observed as they migrated in a fairly straight line toward a small wound produced by a needle previously dipped in croton oil. Sometimes the cells, instead of stopping at the periphery of the wound, turn at its edge and migrate medially. Two histiocytes were observed moving away from a wound. Migration toward a wound begins in 30 or 40 minutes after wounding. Leucocytes also begin to stick to capillary epithelium and to emerge from capillaries.

Histiocytes in tails of 41 tadpoles did not migrate to croton oil wounds when 5 $\times$ 10⁻⁵ gm of pyribenzamine was added per 100 ml of anesthetic solution. The histiocytes do not remain quiescent; cytoplasmic granules are agitated and small

pseudopodia continuously appear and disappear on all sides in contrast to the regular anterior extension of pseudopodia of cells attracted to wounds in the absence of pyribenzamine. The possibility is considered that the cells are stimulated by the antihistamine but not asymmetrically as in a diffusion field.

The number of leucocytes collecting around wounds in the presence of pyribenzamine is decreased and healing time is roughly doubled.

¹ This work has been aided by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

Cytology

142. EFFECTS OF VITAMIN B₁₂ DEFICIENCY ON THE CYTOPLASM OF RAT TISSUES.
George B. Seigel and Leonard G. Worley, Brooklyn College.

Studies have been made of liver, pancreas, and motor neurones of 51-53-day-old albino rats reared on the Zucker type B₁₂ deficient diet and whose mothers, after pregnancy had been detected, were fed a similar diet. The most noticeable cytological effects were on the liver and pancreas. In the cells of the liver, the large basophilic granules of the cytoplasm were greatly reduced in size and number and were almost totally absent in some cells. The granules were most deficient in the peripheral cytoplasm of these cells; such as remained were lodged close to the nucleus. There was a general reduction in the amount of basal chromophilic substance in the alveolar cells of the pancreas of vitamin B₁₂ deficient animals. In neither tissue was any appreciable effect apparent on the size or condition of the nucleolus. Less marked effects on cytoplasmic basophilia were evident in the giant motor nerve cells of the spinal cord. These cytological observations are interpreted as indicating a reduction in the ribonucleic acid (RNA) content of these B₁₂ deficient tissues in view of the fact that in these organs the cytoplasmic basophilia can be abolished with cold (5°C.) 10% perchloric acid, following the method of Erickson, Sax and Ogur ('49). Furthermore, using Schneider's method, the RNA values obtained for B₁₂ deficient liver were approximately 10% lower than those found for normal liver tissue on a wet weight basis. These results tend to support the view that vitamin B₁₂ is of significance in the maintenance of the normal RNA content of these tissues.

143. STUDIES ON DEGENERATING SEX CELLS IN IMMATURE MAMMALS. II. DETAILED DESCRIPTIONS OF THE DIFFERENTIATION OF THE MALE GERMINAL EPITHELIUM IN WHITE RATS AGED 14-62 DAYS AFTER BIRTH AND OF THE ACCOMPANYING TYPES OF PHYSIOLOGICAL DEGENERATION. Ezra Allen and Paul D. Altland, John B. Stetson University, and Experimental Biology and Medicine Institute, National Institutes of Health.

After complete degeneration of the so-called primordial germ cells 10 days after birth the remaining layer of mother cells multiplies for three days and develops two layers, with no well-marked differentiation of either definitive germ cells or Sertoli cells. At 14 days spermatogonia are differentiated, identifiable by their special mode of division. Some of their daughter cells develop on the

same day into preleptotene and a few leptotene first spermatocytes, which are scattered loosely in the central plasma. By the 18th day these cells have increased in number and arranged themselves in two layers surrounded by a few scattered zygotene cells along the developing lumen. Each maturation phase follows the same plan, until at 39 days a thick layer of spermatids is formed, in which a few spermatozoa are beginning to form heads with immature tails. Not until 48 days have the increased number of spermatozoa completed their differentiation and have migrated to the lumen for discharge. At 56 days mature spermatozoa appear in epididymis; at 62 days in ductus deferens. From 39 days to 62 days the number of heads per Sertoli nucleus has increased from 1 to 7 or 8. The first appearance of Sertoli cells has not been determined.

The types of degeneration are ex-foliation, nearly or complete loss of the epithelium, multinucleate, giant, and different types of pycnotic cells. Their causes and functions are discussed. One or more of the types may be present at every developmental stage in some tubules.

144. HISTOCHEMICAL STUDIES ON THE AREA POSTREMA.¹ Walter L. Hard and Richard K. Hawkins, University of South Dakota, School of Medicine, Vermillion.

The area postrema in the medullary floor of the fourth ventricle can be characterized as a highly vascular condensation of neuroglia, and is relatively free of nervous elements. Our attention has been directed to the area as a result of the following histochemical observations carried out on the brains of both dog and cat.

Alkaline phosphatase is limited to the endothelium of the arterial and capillary beds while the large venous sinuses, characteristic of this area of the brain, are negative. This variation in the reaction suggests a physiological differential existing between the arterial and venous beds. Acid phosphatase is intensely concentrated in the area with some tendency of localization in glial cells at the periphery of the blood vessels without distinction to arteries or veins.

Intense reactions for a choline esterase, hydrolyzing myristoyl choline, have been localized in this area. Like acid phosphatase there appears to be some tendency for cells near the vascular elements to exhibit greater activity. These cells are probably identical with those exhibiting an acid phosphatase reaction. The entire area is sharply delimited from adjacent nerve tissue by virtue of its intense reaction.

Countless other areas of the brain and cord have been examined but none reveals sites which exhibit simultaneously the high reactions for all the enzymes reported herein. The high concentration of such biochemical entities would suggest a significant physiology for the area. Further studies are in progress to better identify this role.

¹ Supported by the Office of Naval Research and the U. S. Public Health Service.

145. THE ACTION OF ALDEHYDE-COUPLING REAGENTS ON THE FEULGEN REACTION.

II. AMPHIBIAN AND AVIAN ERYTHROCYTE NUCLEI.¹ M. A. Lessler² and M. J. Kopac, Department of Biology, Washington Square College of Arts and Science, New York University.

Amphiuma, Necturus, Rana, and Gallus blood smears were fixed (6% formalin in 50% ethanol), washed, and subjected to a modified Feulgen procedure. These preparations were exposed to aldehyde-coupling reagents (Na bisulphite, semicarbazide HCl, phenylhydrazine HCl, hydroxylamine HCl or trimethylaminoacetohydrazide) either before or after 10 minutes' hydrolysis in 1 N HCl at 55°C. The degree to which the aldehyde-couplers inhibited the Feulgen reaction was indicated by changes in intensity of coloration in the erythrocyte nuclei.

Sodium bisulphite produced no inhibition in Gallus or Amphiuma nuclei, only a slight inhibition in Necturus nuclei, and a moderate inhibition in Rana nuclei. The semicarbazide produced no inhibition in Gallus nuclei, however, the nuclei of Amphiuma, Necturus, and Rana showed a striking inhibition. Phenylhydrazine produced a striking inhibition in Gallus nuclei, a moderate inhibition in Amphiuma and Necturus nuclei, and only a slight inhibition in Rana nuclei. Hydroxylamine inhibited the Feulgen reaction almost entirely in all four types of nuclei. Trimethylaminoacetohydrazide produced only a slight inhibition in Gallus nuclei while in Amphiuma, Necturus, and Rana nuclei, the inhibition was almost complete.

These differences in the inhibition of the Feulgen reaction in the various nuclei tested are believed to indicate quantitative and/or qualitative differences in reactivity of the hydrolyzed nucleic acids with aldehyde-couplers.

¹ These investigations were supported by Grant C-843, National Cancer Institute, U. S. Public Health Service.

² Post-doctorate Fellow, National Cancer Institute, U. S. Public Health Service.

146. EFFECTS OF RADIO-PHOSPHORUS (P^{32}) ON MITOSIS IN LARVAL TAIL-TIPS OF AMBLYSTOMA PUNCTATUM. Donald P. Costello and Catherine Henley, University of North Carolina.

Young larvae (of appropriate age) of *Amblystoma punctatum*, from which tail-tips had been clipped, were permitted to degenerate in media containing various concentrations of radio-phosphorus. The exact dosage of beta radiation within the epithelial cells of the regenerating tail-tip cannot be calculated, but a sufficient range of dosage was used to give variations in effects on mitosis from zero to complete inhibition. Submaximal dosages produced a wide variety of mitotic abnormalities, some types of which have never before been observed by the authors with other types of treatments. Many deletions (production of chromosome fragments) resulted, similar to those induced in classical plant material, such as *Tradescantia*, by irradiation. Several extreme multiple deletions were obtained, showing 30 or 40 or more chromosome fragments within a single cell.

There was a paucity of multipolar mitoses among the abnormalities produced by the irradiation. Multipolar mitoses are among the most common of the mitotic anomalies produced by temperature effects.

The demonstration that *Amblystoma punctatum* larval tail-tips are a favorable material (comparable, in some respects, to *Tradescantia*) for the study of the effects of radio-isotopes on cells and mitosis is of considerable importance. Investigations of the effects of radiations on animal cells have lagged considerably behind similar work on plants, due largely to a lack of suitable material. Tail-tips of 580 treated larvae have been studied to date, and adequate numbers of controls.

Ecology

147. ON BEHAVIOR OF OYSTERS TRANSFERRED FROM LOW TO HIGH SALINITIES.
V. L. Loosanoff, U. S. Fish and Wildlife Service, Milford, Connecticut.

Groups of adult Long Island Sound oysters were kept for different periods, sometimes several weeks, in water of low salinities (between 3.0 and 10.0 parts per thousand) and then transferred directly to water of high salinity (about 27.0 parts per thousand). The mortality that did occur after the transfer was negligible and usually did not exceed that of the control group. In general, all the oysters survived the transfer unless they were weakened by long exposure to low salinity where many of them kept their shells closed and did not feed, as usually happened in a salinity of about 3.0 parts per thousand or less. The mortality observed in such cases was not due to the rapid change from low to high salinity, but to tissue starvation which occurred prior to the transfer.

In certain experiments some of the oysters kept at low salinity for several days were transferred to high salinity directly, while other groups were brought to the same high salinity gradually, by passing through several intermediate salinities. The mortality in both groups was low and no significant difference between the groups was noted.

Often the oysters transferred from the low salinity of about 3.0 parts per thousand to a high one formed crystalline style, fed and expelled true feces within the first day of transfer. In the spring, when the temperature was rapidly increasing, the oysters transferred from a low salinity to a high one (27.0 parts per thousand) proceeded with normal development of gonads. In general, the observations indicated that oysters are capable of withstanding sharp changes from low to a high salinity without experiencing serious physiological injuries.

148. NON-FEEDING ATTACHMENT OF THE SEA LAMPHREY (*PETROMYZON MARINUS*).
Charles W. Creaser, Wayne University and University of Michigan Biological Station.

The sea lamprey, like other parasitic lampreys, feeds by attaching itself by its rasping mouth to the sides of fishes. However, its less well known habit of attaching itself to other living and nonliving objects is sometimes misunderstood. Observations show that it will attach to such objects as rowboats, and their

oars, motor boats, and humans. Racing shells, like those to which it attaches in Cayuga Lake, often travel up to twelve miles per hour. In our experiences at Burt Lake, Michigan, it has been observed to overtake and attach itself to motor boats going at a rate up to fifteen miles per hour. Such attachments may help to account for its recent distribution through the Welland Canal, around Niagara Falls, and through other rivers of the Great Lakes. The sea lamprey also attaches to human swimmers, according to reports from marathon racers in Lake Ontario at Toronto, and swimmers in Burt Lake and Crooked Lake in northern Michigan. Our observations show that they do attach to humans but do not try to feed. They release their hold when the swimmer leaves the water, leaving only a tooth pattern on the unbroken skin. Several false reports of feeding on humans have been assigned to leeches. When the lamprey is spawning it attaches its mouth to stones and moves them in nest building. It probably seldom rests without being attached to something living or nonliving.

149. RADIAL AUTUMNAL MIGRATION OF THRUSHES IN GASPÉ, QUEBEC. Stanley C. Ball, Yale University.

Hermit and olive-backed thrushes nest abundantly on the Gaspé peninsula. Nocturnal autumnal migrants, readily traced by loud, distinctive notes, become especially vocal in the dawn twilight. From the central highlands radial migration down valleys leads northward to the St. Lawrence, eastward to the Gulf, and southward to Chaleur Bay. Weak upstream flights have been recorded only on the northerly flowing St. Anne and Fox rivers, and on a southern tributary of the Madeleine. Evidence confirms southward, but not northward, crossing of divides between York and St. John rivers; probably Madeleine to York, and St. Anne to Little Cascapedia. A few gray-cheeked thrushes ascended the St. Anne in 1948; many descended the Grand Cascapedia in September, 1949—possibly *Hylocichla minima bicknelli* from breeding grounds in the higher Shickshock mountains.

Near the coast most migrants closely follow indented shorelines. Higher-flying birds cross promontories circumnavigated by lower migrants unable to see beyond.

Data confirm flight eastward along the St. Lawrence shore beyond Grand Étang, westward from St. Anne des Monts, and southward through the Mata-pedia valley.

Several years' observations establish migration on the St. John, York, and Dartmouth watersheds in numbers increasing down tributaries and main rivers. Territory counts suggest total autumn populations equivalent to migrants calculated from records. Evidence discounts heavy migration southward to Gaspé across the wide lower St. Lawrence River.

Simultaneously radial migration contraindicates light intensity, wind, barometric pressure, and southward "urge" as directive stimuli. Descending valley sky-lines may exert an effect.

Consideration is given to the time and distance of thrush flight, robin migration, and routes outside of Gaspé.

Embryology

150. EFFECTS OF REMOVAL OF ECTODERM ON THE ORIGIN AND DISTRIBUTION OF FEATHER GERMS IN THE WING OF THE CHICK EMBRYO. John W. Saunders, Jr., and Paul Weiss, University of Chicago.¹

The location of a prospective feather tract is first signalled in the embryo by the appearance of a series of sub-epidermal condensations of mesenchyme, from which the dermal papillae of the feathers originate. Proliferation of the ectoderm over each papilla subsequently forms the epidermal collar from which the feather arises. To test whether dermal papillae will form in the absence of a covering layer of epidermis, most of the ectoderm was excised from the dorsal surface of the wing bud of the 3-day embryo. This operation resulted (7 out of 16 cases) in the persistence of uncovered mesoderm until late embryonic or post-hatching stages. Histological examination showed that the prospective dermis organized neither a typical corium nor dermal papillae.

The healing of epidermal wounds apparently involves the spreading of the epidermal sheet over the denuded mesodermal surface, a process which conceivably could affect the arrangement of feather papillae. This possibility was tested in the humeral feather tract, where the feather germs emerge in a characteristic pattern, that of a series of intersecting coordinates. Excising small pieces (ca. 0.2 mm²) of ectoderm lateral to the future humeral area in 4-5 day embryos resulted (4 out of 20 cases) in a distortion of this coordinate pattern in the direction of the wound.

These results indicate that the mesoderm is incapable of forming a typical corium in the absence of the ectoderm, and suggest strongly that both ectoderm and underlying mesoderm act conjointly in initiating the formation of feather papillae.

¹ Work initiated at The Johns Hopkins University, and continued at the University of Chicago, where it was aided by the Abbott Memorial Fund and by the American Society through the Committee on Growth.

151. THE EFFECT OF TYROSINE ON THE PIGMENTATION OF EMBRYONIC CHICK SKIN CULTURED IN VITRO. Floyd E. Morbeck and John W. Saunders, Jr., Marquette University.

Skin isolates from the back and saddle areas of 6½-7½ day Barred Rock embryos show an essentially normal differentiation of pigment cells and morphogenesis of feather germs during 4 to 5 days of culture at 37°-38°C. in the saline-agar yolk-albumen extract medium of Spratt ('47). Tyrosine, thought to serve as a substrate for melanin formation in some plant and animal tissues, was added to this medium to ascertain its effect upon the rate of differentiation of melanoblasts (prospective pigment cells). Cultures incubated for 24 hours in the presence of tyrosine (16 milligrams percent) show more numerous expanded melanophores containing darker pigment granules than do control explants of the same age (47 out of 54 cases). Conceivably, the availability of tyrosine may affect the rate at which pigment cells differentiate and synthesize melanin *in vitro*. However, rough measurements show that in approximately 35 percent of the cases, the

feather germs in the isolates supplied with tyrosine are larger than those of the controls. This suggests, therefore, that tyrosine added to the medium might not enter directly into the synthetic reactions leading to melanin formation, but may exert its effect indirectly by way of affecting the rate of growth and differentiation of the feather germs.

152. THE CONTROL OF RECONSTITUTIONAL DEVELOPMENT IN *DUGESIA DOROTOCEPHALA* WITH PILOCARPINE. Olin Rulon, Northwestern University.

When transverse pieces (1/8th the length of whole 14-16 mm planarians) were exposed to certain concentrations (M/500-M/15,000) of pilocarpine nitrate the head frequencies of the reconstituting forms were increased but when the parent animals were exposed to the agent (M/500-M/1000) and the pieces allowed to reconstitute in tap water the head frequencies were decreased (in 7 out of 8 cases). When shorter pieces (1/16ths) from the level just posterior to the head were treated with pilocarpine (M/1000) the scale of organization was increased but when the parent animals were treated before section the scale was decreased. Short posterior pieces (1/16ths) with 24 hours delay of posterior section showed a decrease in scale of organization when treated with M/1000 pilocarpine before section, during the interval between anterior and posterior section, and during the interval between sections plus 48 hours after posterior section. Similar short posterior pieces, when treated with pilocarpine for only 48 hours after posterior section, showed an increased scale of organization as well as a loss of correlative factors as evidenced by the development of a high percentage of bipolar forms. Posterior halves reconstituting in M/1000 pilocarpine developed heads more slowly and with less new tissue than those reconstituting in tap water.

In the concentrations used, pilocarpine was definitely an inhibitory agent. It affected differentially the head-forming tissue and certain correlating mechanisms (present in the intact form and arising at the time of section) associated with the gradient system of the animal.

153. ON THE INITIAL FUNCTION OF THE CHICK THYROID GLAND WITH THE USE OF RADIOIODINE (I^{131}). Louis A. Hansborough and Mustapha Khan, Howard University.

Radioiodine (I^{131}) was injected into the air-chamber of fertilized hens' eggs with the purpose of determining the initial secretion of the labeled iodine-containing colloid in the follicles of the thyroid gland of the embryo. Each of 250 eggs was given an injection of an iodine solution with an activity of 7 microcuries. Observations were made on embryos of five to fifteen days of incubation.

Autoradiographs prepared from whole and sectioned thyroids gave positive results for embryos from the 11th to the 15th day. Thyroids from embryos of five to ten days of incubation left no blackening on the photographic film. Since the appearance of colloid in the follicle is taken as evidence of its secretory function, this indicates that functioning of the thyroid begins on the 11th day

of incubation. A study of microphotographs shows that this is also the time that discrete follicles first appear.

It was further observed that the activity of the gland increases with the age of the embryo. This increase is correlated with the increase in the size of the gland, the number of follicles formed and the quantity of colloid stored.

154. OXIDATION-REDUCTION POTENTIALS OF CHICKEN EGGS. Wasley D. Yushok and Alexis L. Romanoff, Cornell University.

Previous observations by Pavlov and Isakowa-Keo (Biochem. Z. 216: 19-27, 1929) show that in the fresh chicken egg, the oxidation-reduction potential is characterized by a shift towards the positive side, and that of the egg yolk seems to be more positive than that of the egg albumen. Their values for the oxidation-reduction potential, as Eh , were 292 mv and 205 mv for the yolk and albumen respectively.

In the course of experimental work on the deteriorative processes in shell eggs preserved by a plastic coating (Food Res. 14: 113-122, 1949) attempts were made to determine the oxidation-reduction potentials of the egg contents.

The measurements on fresh hens' eggs were made by means of a Beckman potentiometer, with platinum electrode, at a temperature of 25°C. With considerable variation in individual eggs, the average values for Eh of the albumen (pH 7.78) was 414 mv and that of the yolk (pH 6.11) was 333 mv. No significant change was observed with increasing age of the egg.

These figures deviate from those of Pavlov and Isakowa-Keo both in magnitude and in regard to the component of the egg with the higher potential. The discrepancies are difficult to explain except on the basis of the extreme variability among individual eggs.

155. TEMPERATURE AND THE O₂-PRESSURE INHIBITION OF THE EARLY DEVELOPMENT OF RANA PIPIENS. Olin E. Nelsen, University of Pennsylvania.

As previously reported, the exposure for 24 hours at a temperature of 7°-10°C. of fertilized eggs placed in a pressure chamber containing ordinary air plus 45 lbs. of added oxygen, effects complete blockage of development at the end of the blastular period. Ordinary air under similar conditions of pressure does not have this inhibitory action.

A series of experiments using the O₂-pressure exposure for 24 hours at a temperature of 2°-4°C. permits a large percentage of eggs so exposed in any single experiment to escape the inhibitory effects experienced at a temperature of 7°-10°C.

A possible reason for the escape from the blocking-effect by some of the eggs while others do not may be due to the time of sperm-entrance and the fertilization changes experienced before the eggs are subjected to the pressure. For example, some eggs may be immediately entered by a sperm when the suspension is added whereas in others there may be a delay.

The difference in the reaction obtained at 7°-10°C. and that at 2°-4°C. may be due to the inactivity of certain enzymes at the lower temperature with the result that the O₂ has little or no effect upon their subsequent action.

156. THE CYTOLOGICAL DISTRIBUTION OF DESOXYRIBOSE NUCLEIC ACID IN O₂-PRESSURE BLOCKED EMBRYOS OF RANA PIPIENS. Charles G. Rosa, University of Pennsylvania. (Introduced by Olin E. Nelsen.)

Fertilized pituitary-induced eggs were subjected to the blocking-effect known to be produced by exposure to three atmospheres of oxygen added to the normal air within a pressure chamber for a period of twenty-four hours at a temperature of 6°–8°C. Such oxygen-blocked embryos were studied cytochemically in relation to normal ones, and it was found that normal and oxygen-blocked embryos have the same distribution of DNA in the nucleus up to late stage 8 (Shumway) as evidenced by their stainability with the Feulgen and methyl green-pyronin stains. After late stage 8, control embryos increase in their nuclear stainability, whereas the oxygen-treated ones progressively decrease in their reaction to the stains while they continue to the end of the blastular period of development when they become totally inhibited in their development.

157. THE WEIGHTS OF THE KIDNEYS AND THE BLADDER IN THE FETAL AND NEW-BORN DOG. Homer B. Latimer, University of Kansas.

The weights of the two kidneys form about 0.3% of the body weight in the smallest fetuses. They increase irregularly at first but after 200 gm of body weight they are more regular and average 1.13%. These percentages plotted on body length are more regular and may be fitted by two straight lines, the pitch of the second being greater than the first.

The levels of the two kidneys were recorded for a part of the series and the right kidney was more cephalad in 65%, varying from "slightly higher" to as much as 3 mm.

No fat was observed in the gross dissection until a body weight of 26 gm was attained. It always appeared first at the hilum and along the lateral borders. Above 150–160 gm of body weight, pararenal and perirenal fat could be distinguished.

Mesonephri were found in all but one of the smallest 59 fetuses. They varied in weight from 3.5 to 20.9 mg. No trace of the mesonephros was found in fetuses with a body weight above 129 gm.

The percentage weights of the bladder were irregular in the smallest specimens, but above 30 gm of body weight, the bladder averaged 0.114% of the body weight. Most of the bladders were partially or completely filled by 5.3 gm of body weight. Above 6 gm of body weight some of the bladders had well developed walls but were empty, the urine evidently having been emptied into the amniotic cavity.

158. RABBIT FETUSES IN THE ABDOMINAL CAVITY. Homer B. Latimer and Paul B. Sawin, University of Kansas and Roscoe B. Jackson Memorial Laboratory.

A 4 year old race A rabbit from the Jackson Memorial Laboratory was found with a hard and somewhat rough mass imbedded in the fat of the greater

omentum. After removal and due fixation in formalin, it weighed 23.3 gm and measured $61 \times 41 \times 17.5$ mm. Through the thin covering membranes of this crescent shaped mass the outlines of the skeletons of two fetuses could be seen with nose-anus lengths of 112 and 114 mm.

After the removal of the membranes, masses of material resembling dried muscle were found in the thighs and other normally well muscled regions. The body cavities were filled with a cheesy, unorganized yellow mass. The skeletons were intact and the osseous and cartilaginous parts easily distinguished. Both of these specimens gave evidence of being resorbed, with the skeletal parts the last to disappear.

Records show that this mother had borne a series of 11 litters. Following the last litter of one live and several dead rabbits on 1/13/49, there were ten unsuccessful attempts to mate her.

Mated again on 2/28; test-mated on 3/19 at which time she gave the typical pregnancy or pseudopregnancy reaction; she should have produced a litter 3/31, but failed. From which of the last two matings those two young resulted we cannot be certain, but from the whining reactions, typical of pregnancy or pseudopregnancy, in 10 unsuccessful attempts to mate her from January to March it seems highly probable that they belong to the earlier, showing that complete resorption had not been achieved in 5 months time.

159. RABBIT FETUSES IN THE ABDOMINAL CAVITY. Paul B. Sawin and Homer B. Latimer, Roscoe B. Jackson Laboratory and the University of Kansas.

A New Zealand Red, weighing about 8 pounds who had previously borne several normal litters gave birth on Jan. 1, 1932, to a litter of two dead but otherwise normal young, following a gestation period of 31 days. She was apparently normally bred on Jan. 10 but in a few days went off her feed, became irritable and pugnacious and gave other indications of being in abnormal health. She was killed Jan. 23 and a fetus, at least several days beyond term and still enclosed in its fetal membranes was found lying on the ventral side of the stomach and partly enclosed by the omentum. There was no odor of putrefaction. The mesenteries were highly vascularized and blood vessels had penetrated these fetal membranes of the head, along the midventral surface of the body and in the region of the hind feet they had grown into the substance of the fetus itself. No placenta was positively identified but there was some decomposed material in the highly vascularized area around the ventral surface of the fetus, which may have been the placenta.

Ectopic pregnancies may be explained in two ways: ova having escaped into the abdominal cavity become implanted and develop, or, the fetus after developing for a time normally within the uterus ruptures through the uterine wall. Reports of ectopic pregnancies are so rare and the subsequent changes so tend to obscure the true nature of the case that any information which may serve to distinguish the two types seems worthy of publication.

160. AN ECTOPIC PREGNANCY IN THE RABBIT. D. D. Crary and Paul B. Sawin, Roscoe B. Jackson Memorial Laboratory.

Upon autopsy of a castor-rex rabbit, a fetus quite hard and black in color was found attached to the outside of the right horn of the uterus by strong adhesions. There was also a strand of fatty membranous tissue extending from it and connected in the pelvic region. The uterus was examined for scars but none was found. Both ovaries had several prominent corpora lutea. The left ostium was flat and not in too good contact with the ovary. The right ostium was flared and one arm lay over the tip of the ovary. The rabbit had been bred five times (1/23, 2/27, 3/18, 4/5, and 4/8, 1950) and had failed each time. She died two days prior to the date when the parturition of the last mating was due. At the time of the last mating she was palpated and a record of pregnancy or presence of resorbed fetus was made. At autopsy, the placenta appeared to have been resorbed, although the resorption had not gone so far but that ears and limbs were still easily discernible. The fetus was probably full term judging from the hardness and the amount of ossification.

The history of previous sterility, together with the placement of the ostium so as to prevent ova from entering the tubes, seems to be evidence of true ectopic pregnancy.

161. RELATION OF BLASTODERM TO PERIBLAST IN EPIBOLY OF THE FUNDULUS EGG. J. P. Trinkaus, Yale University.

The relative rate of epiboly of the blastoderm and periblast (underlying syncytial layer) was studied in normal blastula and early gastrula stages of *Fundulus heteroclitus* by means of camera lucida tracings. In early blastulae the periblast extends beyond the blastoderm an average distance of approximately 0.2 mm. Flattening and epiboly of the blastoderm then begins. While the blastoderm is spreading over the periblast, the periblast continues its epiboly over the yolk. Nevertheless, by early gastrula stages the blastoderm has spread to approximately 0.02 mm from the periblast margin, indicating that the blastoderm has a greater rate of epiboly than the periblast, at these stages. These observations do not support the hypothesis that epiboly of the blastoderm and periblast (at least in early stages) is caused primarily by contractile tension of the surface gel layer of the yolk sphere.

The blastoderm is attached marginally to the periblast by the thin epiblast ("Deckschicht") and its surface gel layer. When this connection is severed during gastrula stages the blastoderm retracts and, after a few minutes, its margin reattaches to the periblast at the point of contact. The blastoderm then "re-expands" over the periblast and finally, under favorable conditions, approaches the margin of the latter, even though epiboly of the periblast has continued. A reattached blastoderm, therefore, spreads over the periblast at a greater rate than the latter spreads over the yolk. Accordingly, the intrinsic capacity of the blastoderm for expansion, when in contact with the underlying syncytial layer, emerges as a possible factor in epiboly.

162. FURTHER STUDIES ON PHOSPHATE TRANSFER IN THE FROG EGG. L. G. Barth and Lucena J. Barth, Columbia University.

Extracts, prepared as described earlier, from frog eggs were found to contain phosphoprotein phosphatase (PPPase), yolk phosphoprotein, and adenosine triphosphatase (ATPase). When ATP is added to such extracts, inorganic phosphate is not liberated as a result of PPPase activity, but is transferred to a new organic linkage. Using extracts from eggs at stages from fertilization to swimming tadpole, it was shown that PPPase and ATPase activities depend upon two different phosphatases, and that transfer of phosphate through this system depends upon amount of ATP available. The effects of metallic ions further distinguishes between components present in the extracts. Manganese increases percent transfer three-fold without altering the splitting of phosphoprotein. Mercury inhibits PPPase activity without changing percent transfer.

The ATP responsible for transfer is bound to the phosphoprotein; excess ATP may be washed out without changing phosphate transfer. The enzymes, which in crude extracts are associated with the black pigment of the egg, may be obtained in clear solution after elution with acetate buffer, pH 4.7. Work in progress is directed toward further elucidation of the nature of the acceptor phosphate compound, and toward localization of components of the system in different regions of the gastrula. It is suggested that mobilization of energy for differentiation may depend upon such phosphate transfer systems, and thus in turn upon respiration and glycolysis whereby energy-rich phosphate compounds are generated.

163. THE CONTROL OF DIFFERENTIATION BY EXTERNAL FACTORS. L. G. Barth and Lucena J. Barth, Columbia University.

Studies on the differentiation of fragments of ascidian stolons and *Obelia* stems were designed to reveal the external factors controlling differentiation. Both forms exhibit the group effect described for *Fucus* eggs. If four ascidian stolons are arranged with one end of each stolon in close proximity to a center and the other end peripherally located, the peripheral end differentiates into a zooid, while the central end forms a stolon. In the case of *Obelia* two stems placed end to end are sufficient to show the group effect. In both forms a gradient of CO_2 applied to the stolon or stem represses differentiation at the end exposed to the higher concentration of CO_2 . In the case of the ascidian stolon, a gradient of urea or uric acid has the same effect as CO_2 . Since a group of developing mollusc eggs or *Fucus* eggs will repress differentiation of a hydranth at the end of an *Obelia* stem adjacent to the group, it is suggested that the group effect can be explained by the accumulation of simple excretory products and does not involve any specific substances. The studies indicate that CO_2 in the concentrations normally produced by living cells is an important factor in the control of differentiation.

164. REACTIVITY OF RHODE ISLAND REDS TO INSULIN AS SHOWN BY INJECTION AND TRANSPLANTATION EXPERIMENTS. Janet F. Rogan, University of Connecticut. (Introduced by Hugh Clark.)

An analysis was made to determine for Rhode Island Red embryos the effect of insulin with respect to leg length, body weight and tissue changes during develop-

ment. Five units of insulin were injected into the yolk of embryos of the following groups: controls, eggs injected at 0, 24, 36, 64, 90 and 120 hours of incubation. All embryos were recovered on the 17th day. Measurements revealed that the average weight of the treated embryos was significantly lower than the average of the control group. However, weight loss did not vary significantly with time of injection. Insulin effected a marked shortening of the long bones of the leg. The incidence of micromelia increased regularly as the incubation age at time of injection increased. Coelomic transplantation operations were undertaken between normal hosts and donors, normal hosts and insulin-treated donors and insulin-treated hosts and normal donors. Embryos were operated upon at 60 hours. Treated embryos used in operations were injected at 24, 36 or 48 hours. Observations made at the time of operation showed that exposure of early embryos to insulin for short periods resulted in abnormal tail regions, modified torsion and flexion and retarded differentiation in 42.3% of the treated embryos. Recoveries made at 17 days of incubation showed that transplantation of normal limb buds into normal hosts resulted in slightly smaller, perfectly formed limbs. Transplantation operations between normal and insulin-treated embryos were unsuccessful. Possible factors causing mortality in operated treated embryos are suggested and experiments are proposed.

165. THE ORIGIN OF THE PARATHYROID AND THYMUS GLANDS OF THE CHICK AS REVEALED BY MARKING EXPERIMENTS. Joanne Schrier and Howard L. Hamilton, Iowa State College, Ames.

The third and fourth pharyngeal pouches of the chick were marked with particles of carbon on the fourth day of incubation, and the marked cells were then traced in transverse sections of the embryos at stages from the fifth to fourteenth days. In the thirty-four cases which have been examined, the carbon was found either inside the parathyroids or the thymus on the marked side or in the mesenchyme immediately surrounding these glands. In only one case a very few particles of carbon were localized inside the tissue of the thyroid; these were clinging to the wall of a large blood-vessel which apparently had carried them into the gland from the adjacent mesenchyme of the marked area.

The primordium of the thyroid divides into two lobes which move laterally and become closely associated with the parathyroid and lower end of the thymus on either side. In many cases the parathyroid or thymus actually becomes embedded in the edge of the thyroid. In such instances there was always a sheath of connective tissue between the thyroid and the other glands, and particles of carbon were found only in the thymus or parathyroid.

It is concluded, therefore, that the thymus and parathyroid glands of the chick are derived from the third and fourth pharyngeal pouches, in accordance with the generally accepted belief, and that the latter do not bud from the primordium of the thyroid as has been suggested in the recent literature.

166. MITOTIC STIMULATION OF *RANA PIPPIENS* EPIDERMIS BY UNDERLYING GRAFTS OF NERVOUS TISSUE AND PANCREAS.¹ Jane Overton, Department of Zoology, University of Chicago. (Introduced by Paul Weiss.)

Larval urodele epidermis responds to small grafts of nerve tissue by local growth and excessive mitotic activity (Weiss, '40; Overton, '48). Extending this test to anuran larvae, no similar response was found at Taylor-Kollros stages I-III. Earlier stages (Shumway, '25) however, reacted positively, with average mitotic indices (no. of mitoses/1000 nuclei) of 8.4, as compared to 0.9 in normal controls, and 0.7 in older stages with grafts. Grafts of thymus, limb bud mesenchyme, liver, mesonephros, and retina produced no local growth; just as liver, cartilage, skeletal muscle, heart and skin had remained without effect in urodeles. By contrast, grafts of pancreas proved as effective as nervous fragments, raising the local mitotic index to an average of 10.1, as compared to 2.0 and 2.9 in samples 2 mm anteriorly and posteriorly (still within diffusion distance from the graft) and to less than 1.0 in controls. Experiments to determine a possible common mitogenic factor in nerve substance and pancreas are underway.

¹ Work under the direction of Dr. Paul Weiss aided by the American Cancer Society through the Committee on Growth and the Abbott Memorial Fund of the University of Chicago.

167. THE DISTRIBUTION OF PHOSPHORUS³² IN THE EMBRYO AND LARVA OF THE FROG. Louis A. Hansborough and David Denny, Howard University.

Fertilized eggs of the leopard frog, *Rana pipiens*, were exposed to solutions of radiophosphorus of different activities (65, 300 and 550 microcuries). The eggs were allowed to develop until the embryos had reached the following developmental stages (after Shumway): mid-cleavage, mid-gastrula, neural tube, muscular response, gill circulation, tail-fin circulation and operculum complete. They were then fixed in an alcohol-formalin solution and prepared for autoradiographic and microscopic studies.

A study of the autoradiographs shows that the turnover of phosphorus³² is greatest in embryos exposed to the most active solution of radiophosphorus and also that it is a cumulative process, increasing with the age of the embryo. The most active areas of the embryo in each of the stages observed are those areas in which the rate of cell division is highest and consequently, the population of cells is largest. This can be interpreted as indicating that phosphorus is being utilized more rapidly in the synthesis of nucleoproteins in those areas in which the differentiation and growth of organ primordia are in progress.

Endocrinology

168. RESPONSES OF *XENOPUS* AND *ALYTES* TO THE ADMINISTRATION OF SOME STEROID HORMONES.¹ Emil Witschi and John Allison, The State University of Iowa.

The most primitive families of the living Salientia, by their reactions to steroid hormones, differ strikingly from the higher Ranidae and Hylidae. It was shown by several investigations that frogs and tree frogs are easily induced to yield 100% male or 100% female lots of larvae, if sex differentiation proceeds under the in-

fluence of either androgenic or gynogenic steroid hormones. In extensive experimental series Witschi and Chang found that not only all the common typical androgens, but also pregnenolone, desoxycorticosterone and progesterone, if administered in adequate dosages, bring about testicular differentiation in genetically female tadpoles of *Rana sylvatica*. Quite to the contrary, androgens in widely varied concentrations were without masculinizing effect on the differentiation of the ovaries of female larvae of *Alytes obstetricans* (family Discoglossidae) and *Xenopus laevis* (family Pipidae). However in *Xenopus*, immediately following metamorphosis, the androgens induce the precocious development of all secondary male sex characters in both sexes. In contrast to the inability of the androgens to interfere with normal gonadal sex differentiation, the estrogenic hormones exert a very decided feminizing effect on the gonad development of genetically male larvae of *Alytes* and *Xenopus*. Apparently the reaction type of these two species is identical with that of *Discoglossus* (Gallien, '48) and very similar also to that of *Ambystoma* (Foote, '41).

¹ Aided by grants from the National Council Research Committee for Research in Problems of Sex.

169. THE BRONCHIAL DIVERTICULA OF *XENOPUS LAEVIS* DAUDIN. Emil Witschi, The State University of Iowa.

In *Xenopus* larvae one month old (stage 25) a diverticulum bulges dorsally from each bronchus into the pronephric chamber (upper celomic cavity). Rapidly enlarging, these sacs fill the fairly wide spaces between the second muscle segments and the caudal surfaces of the cranium on either side of the condyles. In larvae of stage 26 (appearance of cartilages in limbs, digits) and older, the diverticula are partly embedded in the upper concavities of the second muscle segments but mainly occupy the spaces left by the rudimentation of the first segments. P. B. Weisz ('45) who noticed the rudiments of these "pouches" in his "third-form tadpole," suggests a hydrostatic function. Our own observations indicate that in addition the diverticula assume the role of *auxiliary acoustic organs*. This conclusion is mainly based on the following observations. (1) The round window of the otic capsule, which in *Rana* faces ventrally, in *Xenopus* stands upright in the caudal wall of the cranium. (2) The bronchial diverticulum applies itself directly to the membrane in the round window. (3) The bronchial diverticula rise from the bronchi where they change from the transverse to the caudal direction; i.e., they occupy precisely the same place as the bronchial membranes of *Ranidae*, apparently their homologues. The auxiliary acoustic organ of *Xenopus* appears to be of a more primitive type than that of *Rana* (Witschi, '49); a columella does not form, but aerial sound waves (of the lung sacs) are provided a direct approach to the round window.

170. CHORIONIC GONADOTROPIN IN MATERNAL AND FETAL TISSUES AT VARIOUS STAGES OF HUMAN PREGNANCY.¹ Joyce A. Bruner, The State University of Iowa. (Introduced by Emil Witschi.)

The distribution and concentration of human chorionic gonadotropin in maternal and fetal tissues and fluids was determined throughout pregnancy by bioassays.

The highest concentration is always in the chorionic placenta, supporting the view that the hormone is produced by the embryonic trophoblast. The pattern of distribution suggests that the chorion releases the gonadotropin into the maternal blood, and that secondarily some of it passes the "placental barrier" and enters the fetal system across the wall of the chorionic vesicle. In the maternal body the highest gonadotropin concentration is in the blood; and the uterine endometrium has a higher content than the other body tissues. Although the fetal body contains relatively small amounts of chorionic gonadotropin they may well be of physiologic significance. The ratio of concentration between maternal muscle and trunk tissue of the fetus ranges from 10:1 to 20:1. The concentrations decrease similarly in all fetal and maternal tissues and fluids from the 11th week until parturition falling to about one-twenty-fifth of their original values. The maternal system is cleared of the hormone after therapeutic abortion or after normal delivery, in the same length of time. The hypophysis discontinues gonadotropin production during pregnancy, but after removal of the gravid uterus, resumes its normal functions within not more than 6 days.

¹ Aided by grants from the National Research Council, Committee for Research in Problems of Sex.

171. LEVELS OF THYROID ACTIVITY WITHIN WHICH REPRODUCTION CAN OCCUR IN THE FEMALE GUINEA PIG.¹ Roy R. Peterson, Barbara Rayner, Mina MacNair Brown and William C. Young, University of Kansas.

Reproduction was studied in a control group of 23 females and in an experimental group of 39 females injected with crystalline thyroxine (0.1 mg every 4 days or 0.15 mg every 3 days). All the control animals were found in heat and 14 were mated with normal males. Thirty-five of the 39 thyroxine-injected females were found in heat and 29 of those found in heat were mated with normal males. In all the course of gestation was normal and living young were born. Three of 14 thyroxine-injected females observed during lactation died, but 11 survived and lactation was adequate for nourishment of the young prior to weaning.

The indices of thyroid activity follows: Oxygen consumption in cubic centimeters per 100 gm body weight per hour averaged 58.5 (49.7 to 66.5) in the 14 untreated females and 92.5 (80.2 to 100.2) in 5 thyroxine-injected females selected for the collection of these data immediately after the litters were weaned. Heart rate averaged 258 (221 to 300) beats per minute in the control females and 334 (322 to 342) in the experimental group. Protein-bound serum iodine averaged 2.2 γ % (0.6 to 3.3) and 7.3 γ % (6.2 to 8.3), respectively.

Within the range of thyroid activity thus indicated, it is clear that reproduction including folliculogenesis, heat, impregnation, normal development and lactation can occur.

¹ Aided by grants from the U. S. Public Health Service.

172. SOME EFFECTS OF TESTOSTERONE PROPIONATE ON THE SEX DRIVE OF CASTRATED MALE GUINEA PIGS.¹ Jerome A. Grunt and William C. Young, University of Kansas.

Experiments were started to ascertain the dosage of hormone necessary to return castrated male guinea pigs to the precastrational level of sexual behavior.

Twelve mature males were tested with estrous females about every fourth day to determine their drive. The average score of ten tests for each male was considered to be the drive. The animals were then castrated and tested weekly. By the tenth week less than 40% of the original drive was displayed.

The animals remained at this level for six weeks at which time eight were given daily injections of 25 γ testosterone propionate/100 gm body weight. The remaining four were kept as castrate controls receiving only sesame oil. By the eighth week of hormonal therapy the experimental animals had achieved a score equal to the precastrational score which they maintained for three weeks when this phase of the work was terminated. Within the control group during the same period there was a further drop to between 16% and 28% of the precastrational drive.

On the basis of the precastrational drive, the experimental males were divided into high-, intermediate-, and low-drive groups. Generally during hormonal therapy the high-drive animals had the highest score and the low-drive animals the lowest. Since all the animals received the same amount of testosterone propionate/gm body weight, a somatic factor which influences the activity of the male hormone is postulated.

¹ Aided by grants from the U. S. Public Health Service.

173. THE DURATION OF VAGINAL OPENING DURING SEXUAL MATURATION IN THE FEMALE GUINEA PIG.¹ Donald H. Ford and William C. Young, University of Kansas.

Attention is directed to the observation that the duration of the vaginal opening decreases as the female guinea pig matures sexually. Beginning about the twenty-fifth day a number of guinea pigs were examined twice daily throughout the first seven cycles. The first vaginal opening in 22 animals occurred at an average age of 40.8 days, range 31 to 51 days. It lasted for 6.3 days, range 3.5 to 13 days. The second opening in 21 animals was of 4.9 days duration, range 3.5 to 7.5 days. The third opening in 21 animals was of 4.0 days duration, range 3.0 to 7.0 days. Thereafter the duration of the opening was more constant. That of the fourth opening in 20 animals averaged 3.8 days, the fifth opening in 20 animals averaged 3.4 days, the sixth opening in 20 animals lasted for 3.7 days, and the seventh opening in 14 females averaged 3.6 days. The range for the last four groups was the same in each cycle, 2.5 to 5.0 days. The significance of this change in the duration of the vaginal opening in the sexually maturing female is being investigated.

¹ Aided by grants from the U. S. Public Health Service.

174. THE RELATION OF THE MALE GONADIAL CYCLE AND GONADIAL GROWTH TO DAY LENGTH IN THE SLATE-COLORED JUNCO AND OTHER FRINGILLIDS. Albert Wolfson, Northwestern University.

Birds were trapped during the fall and spring migration and subjected to gradually increasing or constant day lengths ranging from 9 to 24 hours, some for as long a period as two years (data in part from Winn, MS).

The results and preliminary conclusions of these experiments are as follows:

(1) Increases in day length are not essential to induce or permit gonadal growth. Constant day lengths are effective, even when as short as 9 hours.

(2) The occurrence of the gonadal cycle is not dependent on changing length of day.

(3) The rate of gonadal growth is closely related to the length of day, but the response of the gonad depends on its initial condition and the length of day used.

(4) The development and maintenance of the gonad in breeding condition in the spring is related to the length of day. The breeding condition, however, cannot be maintained continuously.

(5) Decreasing length of day *per se* does not induce gonadal regression unless the day length is reduced below the minimum requirement for the stage involved.

The results of these experiments and the preliminary conclusions support the theory (Wolfson, 1947) that the total amount of light received rather than increasing day length *per se* is the crucial factor in regulating gonadal cycles. They also suggest possible regulation of breeding cycles in tropical birds by day length and the regulation of the annual stimulus for migratory behavior by day length in equatorial and transequatorial migrants.

175. GONADIAL RESPONSE TO INJECTIONS OF TESTERONE PROPIONATE IN THE MALE ENGLISH SPARROW AND WHITE-THROATED SPARROW. Albert Wolfson and Helmuth A. Stahlecker, Jr., Northwestern University.

Two experiments were performed. The first began January 12 and was concluded February 4, 1949. During this period a small group of male English Sparrows and White-throated Sparrows was subjected to natural conditions of day length and received 21 daily, intramuscular injections of 2.5 mg of testosterone propionate (Perandren, Ciba). In the English Sparrow the experimental treatment induced gonadal growth to the breeding condition, but the White-throated Sparrow showed no gonadal response. Since the results of this preliminary experiment differed from those of Pfeiffer, a more extensive experiment was designed to determine whether testosterone could induce gonadal recrudescence in initially inactive testes in the English Sparrow.

This second experiment began January 29, 1950 and ran for three weeks. Only English Sparrows were used. The birds were kept under 8 hours of day length from the time of capture in December to the end of the experiment. This period of day length was reported by Bartholomew as being inhibitory to testicular growth in the English Sparrow. The same dosage of Perandren was injected and for the same period of time as in the previous experiment. The results demonstrate clearly that testosterone induced gonadal growth to breeding condition in testes that were initially at the winter minimum.

The results of these experiments and of related experiments in this and other laboratories raises the question of the role of the gonadotrophic factors of the pituitary in regulating the breeding cycles of wild birds.

176. THE EFFECTS OF ADMINISTRATION OF ANTITHYROID COMPOUNDS TO TURTLES.¹

A. Elizabeth Adams and Miller Craig, Mount Holyoke College.

Thiourea and thiouracil were administered to adult painted turtles, *Chrysemys picta*. In series I, 11 animals (autopsy weight, 64.2–78.4 gm) were injected subcutaneously daily (dose, 1.0 cc) for 13–41 days with 0.1, 0.2 or 1.0% of thiourea or thiouracil or both. Three animals were also given thiouracil simultaneously by mouth. Total doses for 2 thiourea-injected turtles were 70.0 and 141.0 mg administered in 41 days; for 7 thiouracil-treated ones, 13.0 (2 animals), 28.0, 34.6, 140.0, 193.0 and 215.0 mg in 13 or 14 doses. Two others received 23.0 mg of thiourea plus either 10.0 or 13.0 mg of thiouracil in 29 or 31 doses. Of 2 controls, one was untreated, the other injected with distilled water.

In the thyroids of 5 turtles there was some reaction. One had received thiourea, 2 thiouracil, and 2 both drugs. Follicular cell height was increased over that in controls and there was some liquefaction and loss of colloid. In the smallest animal given 140.0 mg of thiouracil in 14 doses, the reaction was very marked; in 2 others, slightly less.

In series II, thiourea was administered orally once weekly in gelatin capsules, the dose being 25.0, 50.0 or 100.0 mg. Total dosages amounted to 475.0 mg (129 days), 500.0 mg (142 days), 700.0 mg (95 days), 1000.0 mg (152 days), 1300.0 mg (88 and 94 days). The thyroids of these animals reacted little. Four were quite hyperemic, 4 showed small localized regions of liquefaction and loss of colloid with somewhat increased cell height.

¹ Aided by a grant from the Committee on Research in Endocrinology of the National Research Council. Thiouracil (Deracil) was supplied through the courtesy of the Lederle Laboratories.

177. VARIATIONS IN THE NEUTROPHILE COUNT AFTER INJECTION OF ADRENAL HORMONES. Kathryn F. Stein, Cynthia Martin and Doris Falk. Department of Zoology, Mount Holyoke College. (Introduced by A. Elizabeth Adams.)

Twenty-one intact mice were injected with ACE in oil (Upjohn), 25 with epinephrine, 13 with sex hormone, and 9 with sesame or peanut oil. Fourteen completely adrenalectomized and 5 splenectomized individuals were similarly treated with epinephrine. Total and differential white cell counts were made on tail blood taken 4–6 hours after injection. ACE in doses which caused a similar or greater decrease in eosinophiles and lymphocytes of intact mice did not produce as extreme a degree of neutrophilia as epinephrine. Even in animals which had been completely adrenalectomized, as indicated by the lack of an eosinopenia after epinephrine, this substance produced a marked increase of neutrophiles in the circulating blood. Neither sex hormones nor peanut or sesame oil had this effect. Since a marked neutrophilia occurred in the splenectomized animals after injection of epinephrine the effect of this hormone is not exerted through the spleen. Preliminary observation of bone marrow smears of intact injected animals indicated a higher proportion of neutrophilic to eosinophilic cells in epinephrine injected mice than in those which received ACE. This suggests a direct effect of epinephrine upon bone marrow. Further studies are in progress on the bone marrow of adrenalectomized animals.

178. ADRENAL ANTERIOR PITUITARY STUDIES IN THE HEREDITARY DWARF MOUSE.¹
Mary E. Haack and Clinton M. Osborn, Syracuse University.

Eighty-six hereditary dwarf mice of the Bar Harbor strain were used in experiments designed (1) to determine whether ACTH is produced in the dwarf anterior pituitary as evidenced in compensatory adrenal hypertrophy in unilateral adrenalectomy and (2) to determine survival time and the effectiveness of replacement therapy in totally adrenalectomized dwarfs. After unilateral adrenalectomy 28 to 36 days lapsed in all instances before the remaining gland was removed for weighing and histological study.

In growing dwarfs, the greater weight of the second adrenal could be attributed to proportionate organ growth associated with the increase in body weight. In older mice which had attained their expected growth, the second adrenal enlarged disproportionately, 38 to 73% heavier than in controls. This is considered a true compensatory hypertrophy and is believed to indicate that some ACTH is produced by the dwarf's pituitary. Histological studies revealed some thickening of the cortex but no appreciable qualitative improvement in its tissues.

A total of 1000 gamma of ACTH injected intraperitoneally in 10 doses (thrice daily) produced adrenal enlargement of 83% in 5 days. These glands showed marked cellular hypertrophy with increased lipid droplets throughout the cortex. This response is small, indeed, in comparison with the effects in normal mice reported by other investigators. It is suggested that the peculiar multiple endocrine deficiencies in the dwarf may account for the limited effectiveness of ACTH.

Untreated adrenalectomized dwarf mice survived only 4 to 6 days but with cortical extract (Upjohn) the mice were maintained as long as six weeks.

¹ This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

179. INDUCED ESTRUS IN THE OVARIECTOMIZED GOLDEN HAMSTER. George K. Armen and Arnold L. Soderwall, University of Oregon, Department of Biology.

Behavioral estrus was induced in ovariectomized female hamsters using Progynon-B (alpha estradiol benzoate in sesame oil), Proluton (Progesterone, in sesame oil), and Progesterone crystals. Varying amounts of these substances were administered to determine the optimal dosages.

Estrus was induced in 98.6% of 58 animals by two conditioning injections of 50 I. U. each of Progynon-B followed in 48 hours by 0.2 mg of Proluton. Forty-seven per cent exhibited estrus of thirty animals receiving only 0.1 mg Proluton after similar progynon conditioning.

Eighty-two per cent of 51 females displayed estrus behavior after two priming injections of 50 I. U. Progynon-B followed in 48 hours by the implantation of 0.1 mg of Progesterone. Of 41 females implanted with but 0.05 mg Progesterone crystals following the conditioning doses only 51.2% displayed typical heat reactions.

Latent periods were three and a fraction hours for both groups but the length of estrus averaged one hour (12½) longer for the crystal-implanted group.

The latent period was dependent upon the time interval from the time of administration of the progestational hormone until the onset of darkness.

The normal female hamsters display post-parturitional heat.

180. MODIFICATION OF MOLTING FREQUENCY IN *TRITURUS VIRIDESCENS* BY ULTRASONICALLY TREATED PITUITARY IMPLANTS. Hugh Clark, University of Connecticut.

Four groups of *Triturus* were created in order to ascertain the site of effect of ultrasonic vibrations shown previously to increase the molting frequency. These consisted of (A) 20 normal controls, (B) 21 normal hosts and normal pituitary donors; (C) 20 normal hosts and ultrasonically treated donors, the pituitary being removed and implanted immediately after treatment; (D) 20 normal hosts and ultrasonically treated donors, the pituitary being removed and implanted in the host 10 days after treatment. Treatment consisted of exposure of the head to 35 watts acoustic for 15 secs; vibrations were generated piezoelectrically, 350,000/sec. Molting frequencies were as follows: (A) 1.4 molts/month; (B) 1.2 molts/month; (C) 1.6 molts/month; (D) 4.5 molts/month. This result is interpreted to mean that ultrasonic exposure of newts produces a gradual and possibly cumulative change in the pituitary, probably with reference to a thyrotropic substance. Cytological studies of pituitary and thyroid are in progress.

181. DESOXYCORTICOSTERONE AND PREGNANCY IN OVARIECTOMIZED HAMSTERS. Myron D. Tedford and Paul L. Risley, Biology Department, University of Oregon.

Pregnant ovariectomized hamsters received desoxycorticosterone acetate (Ciba) in doses ranging from 0.5 to 5.0 mg daily from the 9th to the 16th day of gestation. Most animals were ovariectomized the 10th day following mating, the 12th, 13th, 14th, and 15th days. If young were not born by the 17th day, the animals were autopsied.

After ovariectomy on the 10th day, living embryos were found on the 17th day in 75% of the cases. However, parturition was never observed, and in some cases the uterus was ruptured. Those receiving only 0.5 mg daily absorbed all embryos in more than 75% of the cases. Daily doses of 0.1 mg to 0.5 mg of alpha-estradiol (Ciba) given concurrently with desoxycorticosterone increased the incidence of resorption to 100%, as occurred when no treatment was given.

Viable young were produced in all cases when daily treatments of 0.5 to 1.0 mg of desoxycorticosterone acetate were given animals ovariectomized after the 12th day. Then on the 12th day, varying results were obtained. Some embryonic malformations were observed in embryos recovered at term.

182. ATTEMPTS WITH ANTI-NEURAL DRUGS TO BLOCK THE EFFECTS OF PLACENTAL GONADOTROPHINS IN THE RABBIT. Charles H. Sawyer, J. E. Markee and John W. Everett, Duke University.

Both human chorionic (PU) and equine (PMS) gonadotrophins induce ovulation in the rabbit, a species in which ovulation ordinarily follows pituitary gonado-

trophin release in response to neurogenic stimulation attendant upon coitus. Kehl and Molina have recently reported that the effects of PU and PMS are blocked by procaine and Neo-antergan, agents with which they claim to have blocked copulation-induced ovulation. These findings imply that the placental gonadotrophins exert at least part of their actions by nervous excitation of the release of pituitary gonadotrophin. The present experiments, employing more than 50 mature female rabbits, have attempted to block the effects of PU and PMS not only with procaine and Neo-antergan but also with anticholinergic, anti-adrenergic and anesthetic agents which we had previously found effective in blocking neurogenic stimulation of the hypophysis in the rabbit and rat. None of the known blocking agents (Dibenamine, SKF-501, atropine, Banthine and Nembutal) was observed to prevent the ovulatory action of either PU or PMS. Nor have we been able to confirm the finding that procaine or Neo-antergan blocks either the effects of placental gonadotrophins or the coital reflex mechanism. Our results lead us to conclude that if the hypophysis is stimulated by placental gonadotrophins their effects are exerted directly on the gland rather than indirectly via the nervous system.

183. THE EFFECTS OF 17-VINYL TESTOSTERONE, 17 CIS-TESTOSTERONE, AND TESTOSTERONE PROPIONATE ON IMMATURE RATS. T. Bennett and H. J. Evans, Syracuse University.

Male and female albino rats received subcutaneous injections of these synthetic androgens starting when the animals were approximately thirty days old and continuing in most cases for thirty days. Organ weights were expressed in relation to body weight and analyzed statistically.

When administered in 2.5 mg daily doses, vinyl testosterone reduced *adrenal weight* 41 percent in intact females and 26 percent in spayed females, but did not significantly reduce adrenal weight of intact males. Daily doses of 5 mg reduced adrenal weight of intact males 23 per cent. This effect of vinyl testosterone agrees with the finding of Lewis *et al.* in respect to females but shows a lesser response in males. When given in 2.5 and 5 mg daily doses vinyl testosterone reduced *testis weight* approximately 80 percent; *cis*-testosterone and testosterone propionate did not change testis weight significantly. With 1 mg daily doses reduction was 13 percent with vinyl testosterone and 18 percent with testosterone propionate.

Comparable reductions of *pituitary weight* followed administration of testosterone propionate and vinyl testosterone.

Two and one-half milligrams doses of vinyl testosterone produced greater reduction of *ovarian weight* (48%) than did testosterone propionate (29%). Vinyl testosterone decreased *uterine weight* of intact females but increased that of ovariectomized females.

(48%) than did testosterone propionate (29%). Vinyl testosterone decreased *uterine weight* of intact females but increased that of ovariectomized females.

Cis-testosterone and vinyl-testosterone showed weak androgenic effects in reducing the size of the thymus and increasing the size of sex accessories. They had no effect on kidney weight.

184. EFFECTS OF ADRENAL CORTICAL EXTRACT ON RENAL FUNCTION IN HYPOPHYSECTOMIZED RATS. W. R. Boss and C. M. Osborn, Syracuse University.

Previous work has shown that the hypophysectomized rat is unable to excrete a water load rapidly and that this deficiency can be partially repaired by injecting hormones of the adrenal cortex. In order to study these phenomena further creatinine clearance determinations were made using 150-200 gram fasted female hypophysectomized rats. All animals were hydrated by stomach tube with two hourly doses of water (3 cc per 100 sq cm of body surface per dose). Thirty-six rats were given 4 ml of cortical extract (Upjohn) in divided doses as a part of the fluid load. Hypophysectomized control rats in equal numbers received 4 ml of normal saline solution. Clearance studies in controls run at 5, 7, 17, 31, 39 and 54 days after hypophysectomy revealed a progressive decrease both in filtration rate and urine flow which stabilized within a month. Following that the filtration rate remained at approximately 50% and the urine flow at approximately 25% of normal values. In all rats receiving cortical extract urine flow was markedly improved but not restored to normal. The deficient filtration rate was improved little if any. In both normal and hypophysectomized animals given extract the ratio of the filtration rate to urine flow, expressed as ml per minute, was 10 to 1. In hypophysectomized control rats it approximated 10 to 0.25. It is seen then (a) that the probable limiting factor which prevented cortical extract from restoring urine flow to normal was its inability to elevate the lowered filtration rate and (b) that the cortical extract exerted its effects by inhibiting the tubular reabsorption of water (as it does in normal and adrenalectomized rats, Boss *et al.*). It is further indicated that some influence from the anterior pituitary, other than the ACTH-adrenal cortical system, is necessary for the maintenance of the normal glomerular filtration rate.

185. EFFECTS OF STEROID HORMONES UPON THE ADRENALS OF FETAL RATS. Ralph L. Kitchell, Department of Anatomy, University of Minnesota. (Introduced by L. J. Wells.)

In 26 fetuses of 19 litters, a pellet of hormone (Ciba, about 1 mg) was placed under the skin *in utero*. Eight were given testosterone propionate, 8 progesterone and 10 estradiol benzoate. Treatment, begun about 19½ days after witnessed coitus, was continued 51-55 hours. Approximately one hour before parturition, fetuses and litter mate controls of same sex were taken by severance of the umbilical cord.

A preliminary microscopic study of stained adrenal sections failed to reveal any conspicuous morphological differences between the experimental and its control. Cytological studies are unfinished.

The volume of the right adrenal was determined from stained serial sections, using the paper-weight method. This volume was divided by the body weight. In the androgen series, this value was greater than that of a litter mate control in 6 of 8 cases. These differences would seem to be significant ($P_t = .03$). In the progesterone and estrogen series, this value was greater than that of its control in 6 of 8 cases and in 9 of 10 cases, but these differences were not statistically significant. In an additional, special control (transferred from uterus to maternal peritoneal cavity), the value was essentially that of an uterine control.

Contrary to certain hypotheses from studies of juvenile rodents, these observations suggest that the hormones had not reduced the volume of the "fetal" cortex. Instead, they suggest that androgen slightly increased the volume. Further observations are necessary to determine whether this effect is a specific one or even a hormonal one.

186. RELATION OF THE THYROID TO THE WEIGHT AND ASCORBIC ACID CONCENTRATION OF THE ADRENAL. Henry H. Freedman and Albert S. Gordon, Department of Biology, Washington Square College of Arts and Science, New York University, New York.

Five groups of young male rats were treated as follows: (1) 0.2% thiouracil in feed, (2) 0.2% thiouracil and 5 gamma d,l-thyroxin/100 gm wt./day, (3) 5 gamma d,l-thyroxin/100 gm body wt./day, (4) thyroidectomized and (5) thyroidectomized given 5 gamma d,l-thyroxin/100 gm body wt./day. The effects of these procedures upon adrenal weight and adrenal ascorbic acid concentration were determined at 1, 2 and 4 weeks of treatment. Total ascorbic acid concentration was ascertained colorimetrically as the 2,4-dinitrophenylhydrazine derivative.

Thiouracil treatment produced a progressive atrophy of the adrenals to 66% of normal weight at 4 weeks, and an initial increase in ascorbic acid concentration followed by return to normal values at 4 weeks. Thyroidectomy resulted in a less pronounced atrophy of the adrenals with the ascorbic acid concentration falling to 79% of normal at 4 weeks. The effects of both types of hypothyroidism were reduced by thyroxin in the dosages employed.

It is suggested that the reduced ascorbic acid concentration 4 weeks following thyroidectomy reflects a decreased rate of synthesis and/or mobilization of the vitamin in the atrophied adrenals, and indicates a greater adrenal inhibition than is obtained with thiouracil. The results lead to the conclusion that adrenal ascorbic acid concentration may be a reliable index of adrenocortical activity only if related to the change in adrenal size.

187. THE ADRENAL AND ERYTHROCYTE FRAGILITY.¹ Herbert Megel and Albert S. Gordon, Department of Biology, Washington Square College of Arts and Science, New York University, New York.

Normal, adrenalectomized and sham-operated female rats, weighing 140-160 gm were employed as donors for red cells and plasma. The adrenalectomized animals were maintained on 1% saline supplied as drinking water. The resistance of the blood to hypotonic lysis is found to increase following adrenal removal. The resistance attains a maximum at approximately 2 weeks subsequent to operation. The effect is not present when washed cells, instead of whole blood, are employed. The resistance of washed cells is increased when they are immersed in plasma and to a greater degree in that of adrenalectomized than of control animals. Incubation of red cells in the plasma of adrenalectomized rats is accompanied by a disappearance of the inhibitory factor from the plasma. The amount disappearing is directly proportional to the numbers of red cells added. Daily administration of

0.5 cc lipoadrenal extract (Upjohn) or 3 mg cortisone (17-OH-11 dehydrocorticosterone acetate, Merck and Co.) restores the red cell fragility of adrenalectomized rats to normal values.

It is concluded that a plasma factor(s) is responsible for the increased resistance displayed by the red cells of adrenalectomized rats to hypotonic lysis. The factor appears to operate through adsorption (and/or absorption) mechanisms.

¹ Supported by a grant-in-aid from the Dazian Foundation for Medical Research.

188. ADRENAL EFFECTS ON GLYCOGEN AND RESPIRATION OF LYMPHOID ORGANS. Fleur L. Strand and Albert S. Gordon, Department of Biology, Washington Square College of Arts and Science, New York University, New York.

Carbohydrate changes within the lymphoid organs (lymph nodes, thymus, spleen), induced by alterations of adrenal hormone levels, were compared to liver glycogen values in female rats. The ability of the various tissues to reduce 2,3,5 triphenyl tetrazolium chloride was used as the criterion for respiration.

Glycogen values of lymphoid organs and liver in sham-adrenalectomized rats are fairly constant over a 4 week period subsequent to operation. These levels drop sharply in the adrenalectomized animal. Lymphoid organ glycogen values are maintained at control levels in adrenalectomized rats treated with adrenal cortical extract (Eschatin, 1-2 cc daily). Liver glycogen is similarly maintained, with a significant increase above control levels at the first week. Adrenalectomized rats treated with epinephrine (200 gamma daily) display a rise in liver and lymphoid organ glycogen which, in the thymus and lymph nodes, exceeds control values. In the dosages employed, epinephrine has greater effect than the cortical extract.

Adrenalectomy results in an increase in thymic weight which may be prevented by both cortical hormone and epinephrine. Liver and lymphoid tissues of adrenalectomized rats show a greater respiratory activity than those of sham-operated animals. Respiration is lowered in these animals by either cortical or medullary hormone. Maximal effects on glycogen and respiration values are produced at the 2nd or 3d week of treatment. By the 4th week the effects of the hormones are no longer apparent.

It is possible that the glycogen of the lymphoid tissues is made available in stress through adrenal cortical mechanisms.

189. ASCORBIC ACID IN THE ADRENAL GLAND OF THE DUCK. M. X. Zarrow and I. G. Zarrow (by invitation), Department of Biological Sciences, Purdue University.

It has been shown recently that the ascorbic acid content of the adrenal gland of the chicken is not affected by cold or epinephrine. This is an outstanding exception to the decrease in adrenal ascorbic acid observed in rats, mice and guinea pigs and consequently it was felt advisable to investigate another species of the same class as the chicken.

Fifty ducks, 18 to 23 days of age were employed in the present study. In the first experiment the birds were exposed to a temperature of 3 to 5°Centigrade for 1 to 3 hours. In the second experiment the ducks were injected with epinephrine

and killed from 30 minutes to 3 hours after the injection. In both experiments the birds were decapitated and the adrenal glands immediately removed. These were quickly weighed on a torsion balance and extracted with trichloroacetic acid. Ascorbic acid determinations were made by the Roe and Keuther method using the Klett colorimeter. Eighteen day old ducks showed a normal adrenal ascorbic acid of 125 mgm per cent. After 1 hour exposure to cold an ascorbic acid concentration of 123 mgm per cent was observed, after 2 hours 115 mgm per cent and after 3 hours 138 per cent. Similarly, treatment with epinephrine did not affect the ascorbic acid of the adrenal gland. It is apparent that in the duck, as in the chicken, the ascorbic acid content of the adrenal gland is not affected by such stressful stimuli as cold or epinephrine.

General Morphology

190. THYMIC INVOLUTION IN MICE OF THREE AGE GROUPS FOLLOWING INJECTION OF CHLORAZOL BLACK E.¹ Christianna Smith and Hilda Markoff, Mount Holyoke College.

Involution produced by intraperitoneal injections of chlorazol black E was studied in the thymuses of mice, 20-30, 80-100, and 280-533 days of age. In each age group the outstanding characteristic was the reduction in numbers of the cortical lymphocytes, most marked in the youngest group. This diminution of lymphocytes appeared first in the subcapsular zone, the most labile region of the thymus. Dye appeared first in flattened capsular cells, next in macrophages in the subcapsular zone and inner cortex. Dye laden macrophages, exploded cells and giant cells were most numerous in the two younger groups. Plasma cells were most numerous in the oldest group and in each age group they were present in proportion to the degree of involution. There were also more mast cells in the involuted thymuses. Very little dye was found in the epithelio-reticular cells or the foamy cells. Cysts were present in thymuses of all age groups, most numerous in the oldest. The thickening of the capsule was due mostly to argyrophil fibers which in cases of extreme involution were frayed, broken and separated by edema.

¹ This study was aided in part by a Public Health Service Grant, recommended by the National Advisory Cancer Council.

191. GLYCOGEN IN THE THYMUS OF THE FETAL MOUSE.¹ Christianna Smith and Martha J. Waldron, Mount Holyoke College.

Thymuses of fetal mice aged 14 days to birth were studied to determine the time of appearance of glycogen in the thymic cells by means of the periodic acid-leucofuchsin technique. Before and during the transition from an epithelial to a predominantly lymphocytic organ, glycogen was contained in the peripheral and in the anterior epithelial cells, the latter in the region of the cervical vesicle. These epithelial cells were often vacuolated. After the transition, glycogen was found, as in the young post fetal thymus, in the small cortical lymphocytes. It was seen first in these lymphocytes in 17-18 day old fetuses. The proportion

of cells containing glycogen increased until just before birth about one-third of the lymphocytes had glycogen in them.

¹This study was aided in part by a Public Health Service Grant, recommended by the National Advisory Cancer Council.

192. PIGMENTAL VARIATIONS IN POPULATIONS OF POCKET GOPHERS (*THOMOMYS BOTTAE*). Lloyd Glenn Ingles, Fresno State College.

A critical analysis of the differences in pigmental factors between two adjacent races of pocket gophers was made near Fresno, California. Specimens from six collecting stations on a 29 mile line, between Fresno and Auberry, which crossed the zone of intergradation were studied with the aid of a Photovolt photoelectric reflectionmeter. A linear discriminant function $z = \lambda_1 x_1 + \lambda_2 x_2$ was set up for each of the two subspecies (*pascalis* and *mewa*) using as variables the length of the hind foot (x_1) and red pelage color (x_2). The λ s in such a function are determined so that the differences between the means of the two races will be maximized relative to the standard deviations within the two races. By using this statistic it is possible to allocate every specimen in the intergradation zone to one race or the other, or to designate it as an intergrade. Within the zone of intergradation never more than 25% intergrades were found present. The zone of intergradation was found to be four miles wide on the line where the samples were taken. Populations of pocket gophers have the red in the dorsal pelage highly correlated with red color in the soil at the various stations. No visible physical reason is known why the intergrading zone occurs where it does. The differences in population densities of the two subspecies is suspected and may be the explanation.

193. THE SYSTEMATIC SIGNIFICANCE OF HAIR STRUCTURE. William V. Mayer, University of Southern California.

In an investigation of the hairs of 392 species and subspecies of California mammals, involving the examination of over 1000 hair samples, it was found to be possible to identify genera on the basis of hair structure in all cases, species in nearly all cases, and races in a majority of cases. Structural similarities usually characterize the hairs of members of a group, but there are instances of radical differences within a systematic category. Hairs of the genus *Neurotrichus* bear a greater resemblance in length, medullary width, and number of strictures to those of the family Soricidae than to those of the family Talpidae, in which they are placed. A close structural similarity exists between hairs of the families Heteromyidae and Geomyidae, and both resemble shafts of the family Cricetidae. Among hairs of the genus *Perognathus*, greater variations occur than occur among the hairs of some families. In the order Lagomorpha the shafts of the family Ochotonidae more closely resemble those of the rodents than those of the family Leporidae. A peculiar honey-combed appearance of the medulla characterizes all members of the Artiodactyla except the genus *Bison*, whose hairs are similar to those of the Carnivora. No easily demonstrable genetic mechanism explains this eccentric distribution of structural characteristics. However, in view of the important part pelage plays in the classification of mam-

mals, it is not unreasonable to assume that the variations in hair structure and the relationships of hairs within a taxonomic group may be of value in determining systematic position.

194. THE MORPHOLOGY OF THE PANCREAS OF THE GOLDEN HAMSTER, *CRICETUS AURATUS*, WITH SPECIAL REFERENCE TO THE HISTOLOGY AND CYTOLOGY OF THE ISLETS OF LANGERHANS. Howard A. Jewell and Harry A. Charipper, Department of Biology, New York University, Washington Square, New York, N. Y.

The head of the pancreas of the non-hibernating, adult hamster is rather diffuse, while the tail portion is compact. No sex differences can be seen in either the exocrine or endocrine areas and no changes occur during the estrous cycle in females. The intercalated ducts, lined with cuboidal epithelium, lead into larger ducts which are supported by a greater quantity of connective tissue. The exocrine cells are arranged in acini, with the zymogen granules lying towards the center of each acinus. The granular secretion in the intercalated and larger ducts is of the exact same nature (size, shape and staining reaction) as these zymogen granules. Basal to the secretion granules, lies the nucleus and then immediately above the basement membrane, the basophilic ergastoplasm. Long, thin, spiral and rod-like mitochondria are found in the basophilic area and immediately about the nucleus. The Golgi apparatus is always found in the supra-nuclear area amidst the zymogen granules.

The islets of Langerhans are usually regular, compact masses of cells. There is a greater concentration of these islets in the tail of the pancreas than in any other portion. It is possible to show, with the Gomori modification of the Mallory-Heidenhain azan stain, that each islets contains A- and B-cells, both of which contain a granular cytoplasm. The A-cells are larger and contain finer granules than the B-cells. There are no D-cells in the hamster islets.

195. SENESCENT HISTOLOGICAL APPEARANCES IN REPRODUCTIVE SYSTEMS OF AGED MALE AND FEMALE HAMSTERS. Arnold L. Soderwall, Gloria Van Brocklin, and Ruby Ramey, University of Oregon, Department of Biology.

Accepting 24.5 months as the normal life span for the hamster, comparative histological examinations revealed the following statistically significant data for senescent animals:

Female: There was no significant decrease in volumes of mature follicles and corpora lutea.

A significant decrease in number of primordial ova, primary and mature follicles and corpora lutea was noted.

There was a decided increase in number of hyalinized structures in the aged ovaries.

A medullary increase and cortical decrease was apparent. The stroma presented a loose appearance in the senile ovary.

Connective tissue increase occurred in the germinal epithelium of ovaries, subepithelial connective tissue of fallopian tube and myometrium of uterus.

Male: Measurements of total tubule diameters did not vary significantly from younger animals but the lumina diameters increased and wall diameters decreased.

Mild to advanced atrophy was observed in about 70% of the tubules. Atrophy appeared first centrally in the tubule, resulting in arrest of the final stages of spermatogenesis.

Fatty degeneration of cells appeared in atrophying tubules. Sloughed cells and debris increased in the lumina.

Collagenous fibers were more pronounced in tunica albuginea, basement membrane, blood vessels, epididymis and vas deferens.

The epididymis maintained a higher epithelium and showed an increase in the number of hyalinized cells. The normally appearing circular muscles were replaced by connective tissue.

General Physiology

196. EFFECTS OF CONSTANT ILLUMINATION UPON THE MAGNITUDE OF THE DIURNAL RHYTHM OF *UCA*. Frank A. Brown, Jr., Margaret N. Hines, H. Marguerite Webb, and Milton Fingerman, Cresap Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass.

Groups of *Uca pugnax* were maintained under conditions of constant illumination of intensities of approximately 2, 10, 25, 40, 100 and 250 f.c. With the use of a standard scale, the average chromatophore state of each group of animals was obtained at three-hour intervals for a period of several days. The average of the differences between the greatest amount of dispersion of the black pigment and the greatest amount of concentration which occurred during each cycle was used as a measure of the magnitude of the diurnal rhythm. It was found that the magnitude of the rhythm was a function of the intensity of illumination, the greatest magnitude being seen in the 2 f.c. group.

When animals maintained at 250 f.c. for several days were placed at 2 f.c., the low magnitude rhythm observed during the period of treatment with high light intensity was replaced by a rhythm of considerably greater magnitude. By maintaining animals at 250 f.c. for periods of one to sixteen days before placing at 2 f.c., it was found that longer periods of high illumination resulted in a lessened increase of magnitude when the animals were placed at the low illumination. This would suggest that concurrent with the direct inhibition of the expression of rhythm by constant illumination of a high intensity there must also be a gradual suppression of the basic mechanism which controls the magnitude of the rhythm.

197. AN INVESTIGATION OF CERTAIN ASPECTS OF CALCIUM METABOLISM OF *UCA PUGILATOR*. J. Bruce Guyselman, Cresap Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass. (Introduced by Frank A. Brown, Jr.)

In a preliminary analysis of the calcium metabolism of *Uca pugilator*, the Versenate-murexide titrational technique was employed. This method, specific for

calcium, is rapid and permits a high degree of accuracy even in low concentrations.

In one experiment, animals were kept in calcium-free sea water for a period of two weeks. Analyses were made on the external media of one group at regular intervals, and on the constituent tissues of another. The outward diffusion of calcium continued throughout a period of ten days, the hepatopancreas becoming practically depleted of this ion after five or six days. The remainder of the calcium came from the soft tissues and from the body fluid itself; the calcium content of the exoskeleton remained relatively constant. Furthermore, an analysis of normally cast exoskeletons indicates a rather constant percentage of calcium. It would seem unlikely that much, if any, diffuses out from the exoskeleton of animals in calcium-free sea water. The hardening process of animals which molted in normal sea water appears to depend little, if at all, on the utilization of calcium from the cast exoskeleton; whether left at the disposal of the freshly molted animal, or removed at the time of molt, the calcium content of the cast exoskeleton was rather uniform.

In a study of the calcium distribution in the tissues of *Uca pugilator* males, early post molts have calcium distributed rather evenly between the appendages and the body. Intermolts, and early premolts have a higher concentration in the appendages.

198. INFLUENCE OF DAYLENGTH ON THE DIURNAL RHYTHM OF BLACK CHROMATOPHORES OF THE FIDDLER CRAB, *UCA PUGNAX*. Frank A. Brown, Jr., Grover C. Stephens and Muriel I. Sandeen, Cresap Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass.

Experiments were designed to elucidate photoperiodic control of *Uca* diurnal chromatophore rhythm. Two groups of animals were placed in light at 100 footcandles for 6 and 18 hours respectively per day and kept in darkness the remainder of each 44 hour period. These conditions were maintained for an induction period of several days after which both groups were placed in constant darkness. Two control groups were placed in constant darkness and the normal August day lengths, respectively. Two such experiments were followed by staging the chromatophores of each group at three-hour intervals. Under such experimental conditions both rhythm-amplitude and duration of the day (dispersed) phase may vary, the frequency remaining constant.

The magnitude of the day phase in animals exposed to 18-hour light rises steadily above normal values during the induction period, falling sharply and maintaining a level less than, or equal to, normal when placed in darkness. The 6-hour animals show an initial sharp drop on entering the induction period, after which the day-phase magnitude rises slowly to attain a level, significantly lower than normal, which is unaffected by the transition to constant darkness. Animals placed directly in darkness from normal conditions arise to re-attain normal values in five or six days after an initial fall.

These observations suggest that light and darkness each have two quite distinct effects, one immediate and the other evident only subsequent to placing the animals in constant darkness. The dual response to light is probably a function of the postulated dual nature of the control of *Uca* diurnal color change.

199. THE PIGMENT OF ECHINISCIAN TARDIGRADES. Jacqueline Massonneau and Raoul Michel May, University of Paris.

The pigment of Echiniscian Tardigrades (*Echiniscus trisetosus* Cuénot, *E. Blumi* Ritchers, *E. testudo* Doyère, *E. quadrispinosus* Ritchers) is included in small spherical lipidic granules which are free in the coelomic fluid, 2 to 5 μ in diameter, and very refractive. These granules move about as the organism moves, and are yellow, orange or red in color.

Dissolved in appropriate solvents, such as carbon sulphide, this pigment gives a spectrum with 3 absorption bands in the indigo region. It is insoluble in water, glycerine, formalin, dilute (1%) acids and alkalis. It is slowly soluble in cold alcohol (2 hrs.) more quickly in chloroform, acetone, toluene, petroleum ether (a few min.) and very quickly in carbon sulphide (10 sec.). It is very slowly oxidized by air oxygen (2 to 3 weeks at 25°C. with light). It is more quickly oxidized by 1% solutions of chromic acid or potassium bichromate—10 min. as a smear, several hours for an entire *Echiniscus*.

Concentrated sulphuric acid gives an intense blue color and then destroys the pigment in less than 10 sec. A solution of iodine in potassium iodide dropped on an *Echiniscus* previously fixed with formalin gives a violaceous brown color.

These reactions are characteristic of carotenoids. The pigment of the above *Echinisci* is thus a carotenoid. It seems to be of alimentary origin, as its intensity does not change during the desiccated state, but augments when they are placed on a Lichen rich in carotenoids, such as *Xanthoria parietina* Acharius.

200. ADAPTATIVE REACTIONS OF TARDIGRADES TO VARIATIONS IN SALINITY. Jacqueline Collin and Raoul Michel May, University of Paris.

Macrobioti (Tardigrada) both "terrestrial" (*Macrobiotus Hufelandi* Schulze) and aquatic (*Macrobiotus macronyx* Dujardin, *Hypsibius megalonyx* Thulin) were immersed in solutions of chlorides (NaCl, KCl, CaCl₂, MgCl₂). When their molecular concentration was superior to the concentration of NaCl 60/00 the Macrobioti became rapidly immobile after a few seconds to a few minutes, according to the concentration. They then took on the "desiccated" form, as when they are deprived of water. In solutions equimolecular to NaCl 60/00 immobilization occurred only after one-half to one hour. There thus seems to be a relation between this phenomenon and the osmotic pressure of the environment.

The Macrobioti can return to active life within certain limits of concentration and duration of immersion. Immersion in sea water is tolerated better than in an equimolecular solution of NaCl: 1 day in sea water is generally supported while 6 hrs. in NaCl 37% renders revival very difficult.

Passage to the "desiccated" form entails an active muscular contraction dependent on the nervous system and a passive desiccation independent of the latter. These two reactions can be dissociated by studying asphyxic *Macrobiotus*.

Our results confirm the hypothesis concerning the marine origin of Tardigrades.

201. SEPARATION OF EXCITATION AND RESPONSE AT THE POSTSYNAPTIC MEMBRANE.

Theodore Holmes Bullock, University of California, Los Angeles and Zoological Station, Naples.

Recent findings in the literature of synaptic models in nerve fibers and on the giant synapses in squid stellate ganglia suggest a useful distinction between excitation and response in the postsynaptic unit. The principal fact is that signs of transmission lability, e.g., facilitation and fatigue are accompanied by depression of response of the postunit without change in transmission time or, apparently, in the presynaptic spike. Thus in fatigue the start of the synaptic local potential need not be delayed, but its rate of rise is decreased and thus the start of the postspike may be delayed. Therefore events up to and including transmitter action and some mechanism we may call excitation of the postunit are unchanging while subsequent processes intracellular in the postunit appear solely to account for these forms of lability.

There is no reason to assume the excitable mechanism trips the response mechanism instantly. It may do so some time before the start of the synaptic potential. Thus some fraction of the synaptic delay may be intrinsic to the postunit, leaving a shorter period assignable to transmitter action.

The suggestion that crucial events *in the* postunit, presumably chemical ones, occur before manifest response begins may explain cases of long synaptic delay, of junctional excitation surviving a period of maximal antidromic activity and the high temperature coefficient of synaptic delay. These casts do not necessarily support the theory of chemical transmitter action as has sometimes been supposed.

202. A CORRELATION OF ANAPHYLAXIS AND PRECIPITIN PRODUCTION IN CHICKENS.

Takashi Makinodan, Harold R. Wolfe and Morris Goodman, University of Wisconsin.

Anaphylactic shock could easily be produced or prevented in chickens by controlling the interval of time between injections. Injections of beef serum and beef albumin showed that the shocking period as correlated with the peak of the precipitin content of blood. The degree of shock appears to be in part a function of the precipitin titer and the quantity of the antigen used in the shocking dose. The period of sensitivity after one injection was from about 6 to 10 days; after multiple injection series the period was approximately from 4 to 12 days after the last injection.

203. RETINAL AND RECEPTOR AREA AND INTENSITY THRESHOLD. Charles Haig, New York Medical College, Flower and Fifth Avenue Hospitals.

At least four equations have been proposed for the relation between homogeneous retinal areas (single locus) and the absolute intensity threshold, viz.: (1) $AI = C$ (Rice, 1877); (2) $A^{0.5}I = C$ (Piper, 1903); (3) $(A - n_r)^k I = C$ (Wald, 1938); (4) $\log I/I_0 = C R^k$ (Graham and Bartlett, 1939), where A is retinal area, I is the intensity limen, R is radius, and C , I_0 , n_r and k are constants. These apply only to homogeneous retinal areas and are limited either to small areas (1) or to large areas (2) (3) (4).

Using the total cross-sectional receptor areas (inner segments), computed from the histological data of Østerberg (1935) and Polyak (1941), the relation $(I - I_m)(A_t - p) = C$ may be shown to apply to non-homogeneous as well as homogeneous areas of all sizes. Here, A_t is the total rod or cone area within the retinal area under examination, I_m is the intensity threshold of an indefinitely large area, and p and C are constants. When I_m and C are put equal to unity, p assumes a value of 1.0 for cone data and of 0.2 for rod data. This has been shown to hold for cones from the central fovea to 18 degrees in the periphery and for rods from 1.5 degrees to 18 degrees in the periphery.

For homogeneous retinal areas the value of p becomes negligibly small. Thus, for data obtained at a fixed locus the relation $(I - I_m)A = C$ applies. Here, A is either the retinal area or receptor area, since at a single locus the ratio between the two is constant.

204. FAILURE OF DL-METHIONINE SUPPLEMENT TO ALTER THE EFFECTS OF HYPOXIA IN RATS. Paul D. Altland and Benjamin Highman, Experimental Biology and Medicine Institute, National Institutes of Health.

The value of DL-methionine in growth promotion, maintenance of nitrogen balance and as a lipotropic agent is well known. An effort was made to determine if 0.5% methionine in the drinking water of rats would affect favorably the altered growth and pathological changes induced by hypoxia. Thirty weanling Sprague-Dawley rats (20 given methionine) were exposed 4 hours daily to a reduced barometric pressure of 282 mm Hg for 10 weeks. The greatly retarded growth and sexual development was unaffected by the supplement of methionine. Daily exposure of 24 adult male rats (12 receiving methionine) to hypoxia for 10 weeks resulted in a 10% reduction in body weight, a 22% increase in hematocrit, and a 100% reduction in testis weight associated with severe testicular degeneration. The methionine did not alter any of these effects of hypoxia.

No differences were noted at autopsy between the rats receiving methionine and the controls. Microscopically, the rats exposed to hypoxia as adults showed changes essentially similar to those reported (Arch. Path., 48: 503, 1949) in rats exposed to hypoxia beginning at 14 days of age. The incidence and severity of the changes, such as myocardial fibrosis, thickening of the mitral valves, renal hemosiderosis and lipid depletion of the adrenal cortex, tended to be greater in the rats receiving the methionine. The incidence of fatty changes in the heart, liver and renal arteries and tubules was not decreased by methionine.

205. EFFECT OF HYPOXIA ON GERM CELLS OF IMMATURE MALE WHITE RATS. Paul D. Altland and Ezra Allen, Experimental Biology and Medicine Institute, National Institutes of Health, and John B. Stetson University.

Three hundred eighty-five newborn Sprague-Dawley rats were exposed 4 hours daily to 25,000 feet simulated altitude. Approximately 70% died prior to 21 days of age. In fifty-seven exposed rats and an equal number of controls examined during this period no significant difference in testis/body weight ratios were found between the 2 groups. The testes of 30 exposed rats and 31 control rats of equal age were examined cytologically at intervals from birth to 42 days of age. In both groups degeneration of so-called primordial germ cells was found to be complete at 10 days. In the specimens older than 10 days no difference in development was found up to the appearance of spermatids at 27 days, when these cells were abundant. At 29 to 42 days the number of spermatids varied in the exposed rats from none to many. Only a few exposed specimens showed spermatids beginning to differentiate spermatozoa from 35 to 42 days, although this stage appeared at 27 days in controls. No spermatozoa heads were found at any stage of development in exposed rats as late as 42 days, whereas in controls spermatozoa heads appeared at 27 days and were well developed spermatozoa at 42 days, though still attached to the epithelium.

The types of germ cell degeneration, as given for controls in a separate abstract in this issue, were unaffected by hypoxia, but the quantity of degeneration was greater in the exposed rats.

206. FAILURE OF PUPATION OF EPHESTIA LARVAE FOLLOWING EXPOSURE TO X-RAYS.¹ Anna R. Whiting, University of Pennsylvania.

The Mediterranean flour moth, *E. kuehniella*, belongs to the group of insects (homodynamic) in which a continuous succession of generations occurs so long as conditions are favorable. In the course of several experiments full grown larvae were irradiated with doses ranging from 40,000 r to 160,000 r. Following a short period of inactivity they moved about normally and were readily stung and fed upon by the parasitic wasp *Habrobracon*. Those not exposed to the parasite continued to crawl and none pupated. All lived for at least a few days and some (41/177 or 23.16%) are still alive thirty to forty days after exposure. Control larvae pupated three days after time of exposure of treated. The behavior of the irradiated larvae resembles that of diapause larvae in forms (heterodynamic) which show a prolonged arrest of growth in this stage. Whether the x-ray-induced inhibition is due to an interference with secretory processes controlling pupation or to injury to cells of the larval imaginal discs vital to pupal formation is not known. Two irradiated in prepupal stage pupated but have failed to close.

¹ Work aided by a grant from The National Cancer Institute, U. S. Public Health Service.

207. CONTROL OF LUMINESCENCE IN PHENGODES. John B. Buck, Experimental Biology and Medicine Institute, National Institutes of Health.

The light organs of the larviform female of *Phengodes* glow continuously for weeks. The organs of the larva may glow steadily for long periods, but may

also become non-luminous. In both forms the intensity of the light emitted increases during mechanical stimulation, and some of the organs may at times show a less intense light than others.

Luminescence and activity of both female and larva are inhibited completely and reversibly by oxygen-free helium, carbon dioxide or carbon monoxide. In nitrogen containing 0.25% oxygen the animal becomes immobilized, but luminescence persists at a level just perceptible to the dark-adapted eye. In nitrogen containing 1% oxygen, locomotion continues and luminescence persists at a level higher than in 0.25% oxygen but lower than in air. Neither pure oxygen nor a current of air alters the luminescence from its normal intensity in air. When air is admitted after hypoxia or anoxia, luminescence quickly reappears and rises to, but, not above, the pre-anoxic level. There is no "pseudo-flash" or "anoxic glow" as in larval and adult *Photinini*.

It is concluded that the female, at least, of *Phengodes* lacks any sort of internal (end-cell or tracheolar) control over luminescence, and that such slight intensity fluctuations as are seen are incidental to changes in overall metabolism or activity. It is further concluded that luminescence is maximal in 20% oxygen or above, and decreases in proportion to the reduction in partial pressure of oxygen below the level in air.

208. CHEMICAL CONSTITUENTS OF THE BLOOD OF THE JAPANESE BEETLE (*POPILLIA JAPONICA* NEWMAN) LARVA. Daniel Ludwig, Department of Biology, University College, New York University.

The blood of the Japanese beetle larva gels almost immediately on being shed. Ether anaesthesia is effective in preventing this gelation. Blood was obtained from etherized third-instar larvae by allowing it to drip into the depression of a porcelain spot plate. It was then measured into 15 cc graduated centrifuge tubes in which protein precipitation, by the addition of 20% trichloroacetic acid, was made. Analyses, except for protein nitrogen, were made on the filtrate. Samples of 0.1 to 1.0 cc of blood were used for each determination depending on the requirements of the procedure. The following results, expressed in milligrams per cent, were obtained: calcium, 31.6; magnesium, 47.1; sodium, 46.5; potassium, 37.2; chlorides, 111.2; inorganic phosphates, 15.2; nitrogen, 1302; protein nitrogen, 793; and non-protein nitrogen, 509. These results are in agreement with the generalizations made by Florkin (Morgulis, 1949, *Biochemical Evolution*) that insect blood is characterized by a high concentration of magnesium and of amino acid nitrogen.

209. IS ULTRAVIOLET PHOTOLYSIS OF PROTEINS INCREASED BY SUBSEQUENT HEATING? Arthur C. Giese, Stanford University.

Organisms are readily sensitized to heat following ultraviolet irradiations, that is, a heat exposure which is harmless to a control will kill an organism exposed to an ultraviolet dosage which is itself only slightly if at all harmful (Bovie). Proteins are sensitized to heat by ultraviolet radiations insofar as they are precipitable at lower temperatures after such treatment than are control solutions

(Clark). Protein denaturation by ultraviolet radiation is thought to involve splitting of the peptide bond which is the weakest bond in the molecule (Pauling and Niemann) and it has been suggested that one might interpret the sensitization of protoplasm to heat by ultraviolet light by assuming that such breaks have occurred but are not injurious until the molecules are disrupted by subsequent thermal agitation which is greater the higher the temperature. The photolysis of proteins was tested by determining the nitrogen which is rendered non-precipitable by 10% trichloroacetic acid, using the Kirk micro-Kjeldahl test for the supernatant. For a series of four experiments with serum albumin the data indicate that ultraviolet light alone resulted in the breakdown of 15.5% of the total nitrogen using dosages of about 10^6 ergs/mm² while after-treatment with one to several hours of heat at 50°C. resulted in 16.0% breakdown of the total nitrogen present. It may therefore be concluded that whereas ultraviolet fragments a considerable fraction of the protein, heat after treatment as applied has little effect. Similar results were obtained with globulin. Caprylate, which protects against the increase in absorption resulting from exposure to ultraviolet radiations, does not protect the serum albumin against photolysis by these radiations.

210. BLOOD PRESSURE MEASUREMENTS ON TWO SHORE CRABS. Daniel Rogers and Arthur C. Giese, Stanford University.

Blood pressure measurements in cm water were made by inserting canulae containing an anticoagulating solution into blood vessels or sinuses. For *Hemigrapsus nudis* an average value of 5.7 cm water was obtained for systole, 5.0 for diastole in the heart. This value rose when the crab was stimulated to move its legs (to almost 8 cm water). Arteries leading directly from the heart had almost the same systolic and diastolic pressures. The ventral sinus showed an average pressure of 0.85 cm and the pericardial sinus 0.5 cm. There is thus a continued decline in blood pressure with increase in distance from the heart. In *Cancer productus* higher pressures were recorded: 7.8 in the heart, 2.0 in the chela. In a contracting leg a pressure of 5.2 cm was recorded indicating the effect of muscular contraction on blood pressure. Because of the delicacy of the vessels measurements were difficult to make, but good preparations in animals of the same size gave fairly reproducible results.

211. DO OZONE AND HYDROGEN PEROXIDE SPLIT PROTEINS? Arthur C. Giese, Stanford University.

Ozone and hydrogen peroxide have often been invoked as agents which cause many of the effects of ultraviolet light upon proteins and protoplasm. The fact that one can usually smell the ozone produced by mercury arcs and sterilamps indicates that ozone is present during irradiations with such devices. Hydrogen peroxide is not likely to be formed or detected, but organic peroxides may be.

One of the demonstrable effects of ultraviolet light upon proteins is their splitting into smaller fragments, an action thought to underlie some of their biological effects. To test whether ozone and/or hydrogen peroxide have similar

effects a 1% sample of serum albumin at pH 7.3 was subjected to 10% ozone bubbled through the solution (protected from foaming by capryl alcohol) in one series and to 10% hydrogen peroxide in another. The breakdown of proteins was tested by determining the nitrogen rendered non-precipitable (NPN) with 10% trichloroacetic acid using the Kirk micro-Kjeldahl method. Ozone treatment for an hour had a slight effect, the NPN rising from the control value of 0.21 mg to 0.61 mg (total N being 7.7 mg/5 cc) even when the ozonation was performed at 40°C. Exposure to peroxide for 8 days at room temperature resulted in an increase in NPN from 0.08 to 0.35. In comparison an hour exposure to ultraviolet light (dosage 9×10^5 ergs/mm²) resulted in a 21 fold increase of NPN from 0.05 to 1.05 mg. It is therefore unlikely that the striking photolysis produced by ultraviolet light is explainable as an effect of ozone or peroxide since they have small effects in large concentration and only traces of these are ever present as a result of irradiation. Ozone increases the absorption of ultraviolet light by proteins in such a way as to suggest the oxidation of aromatic amino acid residues in the molecule, therefore the action of these agents appears to be of a different sort than the photochemical splitting produced by ultraviolet light.

212. EFFECT OF CHEMICALS ON CILIARY ACTION IN *Volsella demissa*. Austin W. Pritchard and Arthur C. Giese, Stanford University.

The effects of various chemicals on ciliary movements in *Volsella demissa*, a San Francisco Bay mussel, were studied by "racing" squares cut from ctenadia, in a petri dish of solution placed over graph paper. Records were made of movement per unit time. At room temperature the rate of movement was 2.9 ± 0.25 cm per minute. Gill fragments stored in a refrigerator moved at room temperature at about the same rate for as long as three days, after which degenerative changes set in. The results of immersing gill fragment in isotonic solutions of single salts instead of sea water were much like those obtained by Gray on *Mytilus*: in NaCl activity soon ceased but recovery was quite complete unless the sojourn was too long; in KCl the effect on activity was less striking, but recovery was much less complete; MgCl₂ was less toxic than either of the monovalent ions, and CaCl₂ appeared to have least effect on activity but recovery was slowest. An isotonic mixture of KCl and NaCl was most toxic of all, while a mixture of NaCl and CaCl₂ had little effect on movement and addition of KCl made a solution in which movement was indistinguishable from that of the control. Dilution to 80% sea water caused an increase in rate of movement but further dilution was deleterious. Increased concentration of salts always retarded movement. A pH of 8.6 was optimal but good movement was observed between 6.4 and 9.3.

Alcohols (methyl, ethyl, propyl, butyl and amyl) depressed ciliary activity, the effects increasing roughly in proportion to penetrability as predicted by Traube's rule. Urethane poisoned the preparation, methyl urethane being most toxic, ethyl least. Cyanide inhibited at a lower concentration than azide and had a more lasting effect than the latter.

213. ON THE OCCURENCE AND SOLUBILITY OF A REFLECTING PIGMENT IN THE EYES OF THE BRACHYURA. Gilbert F. Gwilliam, University of California, Berkeley. (Introduced by Ralph I. Smith.)

The eyes of the grapsoid crab *Hemigrapsus oregonensis* exhibit a marked "glow" when illuminated in the night-adapted state, and show a white reflecting pigment when dissected in the fresh condition. However, this pigment disappears in the course of routine paraffin embedding and sectioning, although that of macrurans remains unaffected by such treatment. Comparative solubility tests on the macruran *Crago* and on *Hemigrapsus* show the reflecting pigment of *Crago* to be relatively quite insoluble in dioxane, ammonium hydroxide (0.1 M), 10% formalin, 10% neutral formalin, Bouin's fixative, and glacial and 50% acetic acid, while that of *Hemigrapsus* is soluble in all of these. Both *Crago* and *Hemigrapsus* reflecting pigments are relatively insoluble in ether, ether-alcohol, dilute HCl, 95% alcohol, and hot water. Sections of *Hemigrapsus* eyes retaining reflecting pigment have been prepared by killing in hot water, followed by 5% HCl in 70% alcohol to decalcify, ether-alcohol, embedding and sectioning in nitrocellulose. This study clarifies two points: (1) the characterization of crustacean retinal pigment as amorphous guanine, based upon the macrurans (Welsh, J. Exp. Zool., 62: 1932) may not be broad enough to cover the variations existing in crustaceans, and (2) the belief that brachyurans lack the retinal reflecting pigment (Kleinholz, Biol. Bull., 72: 1937, and others) may be based upon an artifact caused by the solution of this pigment during histological manipulation.

214. ESERINE-VERATRINE ANTAGONISM IN MUSCULAR ACTIVITY.¹ Alexander Sandow and Geraldine Kiebel. Washington Square College of Arts and Sciences, New York University.

We have studied isotonic mechanical responses of curarized frog sartorii treated with various temporally-ordered combinations of veratrine sulphate (10⁻⁴%) and eserine sulphate (0.00%) and in all cases, stimulated with maximal twitch shocks at 5 minute intervals. With veratrine alone each response exhibits typical phases: initial tetanus (2-3 seconds) followed by a large, decreasing, prolonged contracture (20-30 seconds). If, any time in such a series, eserine is added to the veratrine, considerable decreases appear in duration and magnitude of these phases, which become greatly pronounced with time so that finally only a simple twitch may result. After equilibration to eserine alone twitches are obtained which are initially almost maximal and then become progressively smaller with no response occurring at about the 15th stimulus. When, within such a series, an eserine-veratrine mixture is applied, for some time in the series each response develops a modified veratrine course, initiated by a twitch-like component quickly followed by a brief though full tetanus and terminating in a quite small contracture. Among the inferences derivable from these results, the following is noteworthy. Eserine presumably curtails the veratrine tetanus and contracture phases by its anticholinesterase activity; and this suggests that the electric features, short series of spikes followed by a prolonged diminish-

ing depolarization, that underly, respectively, the veratrine tetanus and contraction, are consequences of some influence of veratrine on the relation between acetylcholine metabolism and excitation-conduction processes.

¹These studies were aided by a contract between the Office of Naval Research and New York University.

215. EFFECT OF VARIOUS AGENTS ON THE METABOLISM OF ETHYL ALCOHOL. F. W. Kinard, W. M. McCord and J. C. Aull, Medical College of the State of South Carolina.

Preliminary to a study of the metabolism of ethanol, an agent has been sought which will increase the rate of disappearance of ethyl alcohol from the blood. Ethanol, 4 gm per kilo body weight, was administered intravenously in fasted dogs. The concentration of alcohol in the blood was determined at hourly intervals for 10 hours.

The blood alcohol concentration of a control series was compared with each experimental series in which various agents were studied. Each of the following failed to alter materially the rate of alcohol disappearance from the blood:

- (a) 30 minutes inhalation of 100% oxygen.
- (b) 30 minutes inhalation of 90% oxygen-10% carbon dioxide.
- (c) 10 gm of sodium pyruvate by stomach tube.
- (d) 50 mg cytochrome C intravenously.
- (e) 0.5-1.0 mg/kg cozymase intravenously.

216. THE RESISTANCE TO NITROGEN NARCOSIS OF INSECTS, SPIDERS, AND PHALANGIDS. W. A. Hiestand, Purdue University.

Different insects, spiders, and phalangids were exposed to an atmosphere of nitrogen (99.98%) in specially constructed tubes and the time required for the last visible movement of the appendages measured to the nearest second. After nitrogen was introduced into the animal chamber it continued to pass through until 400 to 500 times the volume of the insect container had washed through. The figures given here represent averages in time (seconds) for narcosis for groups of 1 to 11 individuals. Surprisingly little difference in survival time was seen in any one species of insect. The greatest variation appeared in the spiders.

Diptera: *Culicidae* 8.5, *Syrphidae* 12, *Tipulidae* 18, *Muscidae* 33. *Hymenoptera*: *Apidae* 10, *Bombycidae* 20.5, *Formicidae* 13. *Orthoptera*: *Acrididae* 22. *Coleoptera*: *Chrysomelidae* 21, *Lampyridae* 45, *Cerambycidae* 119. *Lepidoptera*: *Geometridae* 66, *Noctuidae* 106. *Ephemera*: *Ephemeridae* 320. *Arachnida*: 627. *Phalangida*: 1032. All above measurements were made at $25 \pm 1^\circ\text{C}$. with specimens captured in Forest County, Wisconsin, $45^\circ 37'$ north latitude. Anoxic resistance is apparently inversely related to metabolic rate in insects.

217. THE EFFECT OF CERTAIN CHEMICAL SUBSTANCES ON THE TOXICITY OF ETHYL ALCOHOL. J. E. Wiebers, F. W. Stemler, and W. A. Hiestand, Purdue University.

The influence of insulin anti-histamine, thiamine HCl, epinephrine, alloxan, and glucose administered previously was determined on the toxicity of ethyl

alcohol injected intraperitoneally in mice. Insulin alone in the doses used caused no mortality in mice but when followed by 48% alcohol (10 ml per kilo) killed from 60 to 100% of the mice. Ten units of insulin followed by alcohol killed 100% of the mice, 1 unit followed by alcohol killed 88% and 0.5 units per kilo killed 60%. Alcohol alone in the dose used killed 62% of mice. All of the substances used except glucose increased the toxicity of alcohol. Glucose decreased toxicity of alcohol by 52 to 100%. Anti-histamine increased toxicity 16%, thiamine 45%, epinephrine 35%, alloxan (given several days previously) 63%. Blood sugar determinations showed the alloxan-treated mice all were diabetic with blood sugars well above 200 mg%.

218. RELATIONSHIP OF DILUTION OF ETHYL ALCOHOL TO ITS TOXICITY INJECTED INTRAPERITONEALLY IN MICE. F. W. Stemler, J. E. Wiebers and W. A. Hiestand, Purdue University.

Nine different dilutions of ethanol in water, from 12% to 96% were injected intraperitoneally into 450 mice, each dilution being made to contain exactly the same amount of alcohol so that the dilution factor alone could be studied. Each injection was computed on body weight. All mice were males so sex factors were not involved. Toxicity was measured by the number of mice killed in 24 hours. Toxicity rose from 0% killed with 12% alcohol to 88% killed with 60% alcohol and then declined with alcohol concentrations above 60%, probably due to the coagulation effect of the high concentrations and resultant poorer absorption. Mice showed a tolerance to alcohol when allowed to drink diluted alcohol *ad libitum*. After 19 days during which time they drank 9.5% alcohol their tolerance increased 59%, so that only 36% were killed by injections of 48% alcohol which normally killed 62% of non-habituated mice.

219. DIFFERENTIATION OF THE REGIONAL ACTION SYSTEMS IN THE URODELE SPINAL CORD.¹ Howard Holtzer, Department of Zoology, University of Chicago, (Introduced by Paul Weiss.)

The development of intrinsic regional coordination systems in the urodele spinal cord was studied by replacing the lumbo-sacral segments (normally innervating the hind limbs) by other regions of the cord in free swimming larvae of *Amblystoma punctatum*, 18 to 25 mm in length. Sensory and motor tests indicated restitution of cord continuity within 2 weeks post-operative.

(1) Substitution by brachial segments in normal orientation resulted, after an initial period of generalized contractions with prolonged after-discharges, in the appearance of remarkably normal and smooth hind limb coordination during withdraw reflexes, swimming and ambulation. (2) Substitution by brachial segments in dorso-ventrally inverted position gave the same results as under (1). (3) Substitution of part of the lumbo-sacral region only by part of the brachial one so that the hind limbs were innervated from mixed sources, yielded persistent mass contractions with long after-discharges without signs of coordination. (4) Upon substitution of the lumbo-sacral segments by thoracic segments the

by Detwiler and Carpenter (1929) on the deficient function of transplanted limbs innervated from trunk segments.

Conclusions: Central coordination systems are regionally differentiated and reside in corresponding cord levels. Hind limbs can be properly actuated by fore limbs segments, even after dorso-ventral inversion of the latter, but trunk segments are intrinsically incapable of acting as limb centers. Innervation from mixed segments produces incoordination. The experiments prove and define more closely the central origin of coordination patterns (Weiss, '41).

¹ Work under the direction of Dr. Paul Weiss aided by the Public Health Service and the Abbott Memorial Fund of the University of Chicago.

220. ERYTHROCYTE COUNT IN RELATION TO MIGRATION AND MOLT IN THE SLATE COLORED JUNCO AND OTHER FRINGILLIDS. Albert Wolfson and Meribeth J. Mitchell, Northwestern University.

Two experiments were performed and involved about 70 birds. In the first experiment birds were trapped during the fall migration and subjected to artificial increases in day length beginning December 3 and terminating in March. During this period gonadal recrudescence occurred, ultimately reaching the breeding condition, and the premigratory change in physiological state was begun and completed. None of the birds had begun the annual molt. In the second experiment the birds were trapped during the spring migration in May and held under natural conditions of day length through October. During this period the gonads reached breeding condition, regressed to the normal winter minimum, and the annual molt was begun and completed.

The range in erythrocyte count for the three species studied was as follows: Slate-colored Junco—3,980,000 to 6,950,000; White-throated Sparrow—3,000,000 to 6,240,000; White-crowned Sparrow—4,120,000 to 6,585,000. The data for the junco show a definite variation in erythrocyte count which can be corrected with migration, molt, and other phases in the life cycle. The highest counts were found in May (normal spring migration) and associated with the premigratory state artificially induced in January. The lowest counts were found in August (normal annual molt) and associated with the period preceding the molt which was artificially induced in late March. The data for the other species, although not as extensive, are corroborative.

Observations on body weight and fat deposition confirm previous conclusions on their relation to photoperiod and migration. The variations in erythrocyte count are correlated with variations in body weight and fat deposition.

221. A RESPIROMETER FOR THE MEASUREMENT OF THE OXYGEN CONSUMPTION OF AQUATIC AND AMPHIBIOUS ANIMALS. Thomas B. Calhoun and Clifford A. Angerer, Department of Physiology, Ohio State University.

Due to the lack of suitable techniques for measuring the oxygen consumption of aquatic animals, a method was sought involving less labor and manipulations, and therefore, less experimental error than the Winkler technique. The method is based on the same general principles as that employed by Scholander and by Vennesland. It consists of determining the amount of decrease in volume of the

potentially closed system necessary to compensate for any decrease in the total atmospheric pressure inside that system, caused by the utilization of oxygen by the respiring animal.

The apparatus, which is immersed in a Warburg waterbath, consists of a main animal chamber to which is attached: (1) a sidearm for CO₂ adsorption, (2) a 1 ml T. B. syringe (accurate to 0.01 ml) with which the measurements are made, and (3) a capillary sidearm which leads to a compensatory flask. On this indicator-containing capillary tube is an index mark. To compare the accuracy of the respirometer with the Winkler technique, oxygen consumption determinations were made on two groups of 18 "normal" goldfish. The results show that the mean metabolic rate and that the standard error of the two groups are essentially identical. An important advantage in favor of this over the Winkler technique is found in the greater linearity of the points of a graph on plotting the ml of oxygen consumed as function of time. This is especially true for animals exhibiting low oxygen uptakes.

222. THE RESPIRATORY METABOLISM OF RED CELLS FROM HYPERTHYROID PATIENTS.

Luis Angelone, David H. Watkins, and Clifford A. Angerer, Departments of Physiology and Surgical Research, Ohio State University.

This study is a continuation of the work from this laboratory on the respiratory metabolism of red cells (Gonzalez and Angerer, 1947; Angelone and Angerer, 1949; Angelone, Morton, and Angerer, 1949).

Blood (35-50 cc) was drawn in the presence of heparin from 6 hyperthyroid patients and 13 "thyroid-normal" patients. The red cells were centrifuged to a constant volume, washed twice, and resuspended in an equal volume of Krebs' Ringer solution (pH 7.4). Aliquot samples were placed into 3 to 6 respirometer flasks for study at 37.5°C. A study of the "normal" cells was made immediately after preparation of the cells by use of the Fenn technique and after over-night storage in the refrigerator (2-8°C.) by use of the Warburg technique. Since no difference in the mean values was noted, the latter technique was used to study the cells from the hyperthyroid patients. The "normal" and hyperthyroid mean QO₂ values were found to be 0.013 and 0.030, respectively; more than 100% increase of the hyperthyroid over the control. This may be interpreted as due to the excess hormone elaborated by the hyperfunctioning thyroid.

223. TWO GLYCOGEN FRACTIONS IN TISSUES OF THE CHICK EMBRYO. Joseph V.

Michalski, Emory University.

Biochemical studies on glycogen levels in liver, heart, and skeletal muscle of chick embryos have been reported (Michalski, 1950). Subsequent studies have been made of the glycogen content of the lungs, smooth muscle of gut, and chorio-allantoic membranes of the chick embryo. Two methods of extraction have been employed: the classical Pflueger KOH method, and extraction with 10% trichloroacetic acid. Determinations were made using anthrone reagent and the Evelyn photoelectric colorimeter.

Glycogen can be demonstrated in the lungs, chorioallantoic membrane, and smooth muscle of gut as early as the 7th day of incubation. There is a gradual, irregular increase in concentration during development, reaching a value of approximately 0.07 gm per cent wet weight on the 18th day for lung and smooth muscle, with slightly lower values obtained for chorioallantoic membranes. In each of these tissues the trichloroacetic acid extractable glycogen is approximately equal to the KOH extraction value from the 7th until about the 12th day. Thereafter, the acid extractable glycogen levels off, or decreases with respect to the alkali extractable glycogen. Bloom and Lewis in their investigations on rat tissues have demonstrated similar relationships in glycogen extracted by these two methods, and have suggested that the acid extractable glycogen is the most physiologically labile of the glycogen present in tissues.

224. HAIR DEVELOPMENT AS INFLUENCED BY PERCUTANEOUS APPLICATION OF CHEMICAL AGENTS. Harold Rauch, University of Massachusetts. (Introduced by Herman B. Chase.)

Areas on the backs of C57 Black mice were painted daily for six days with ether, benzene, or concentrated aqueous K_2HPO_4 solution. The areas selected for such treatment were characterized by the presence of hair follicles in either the anagen (active) or the telogen (inactive) phase of the development cycle. Although treatment with ether or benzene during the active phase produces marked alterations of the integument, including thickening, inflammation and scab formation, hair development is unaffected. The rate of development and the form of the final product are normal. Similarly, development after treatment with the phosphate solution during this phase proceeds normally. Treatment with any of these agents during the inactive phase results simultaneously in epilation of the completed club hairs and the immediate initiation of the development of the next hair generation, which then succeeds in a normal manner. Histological observations indicate that both the epilation and the initiation of development are primary effects and are not dependent upon each other.

It is concluded that topical applications of ether, benzene and K_2HPO_4 solution are effective only during the telogen phase. The result is the premature initiation of the development of the next hair generation. Treatment has no effect on the rate of development nor the form of the final product.

225. THE INFLUENCE OF WATER TEMPERATURE ON TIME OF SURVIVAL AFTER SUBMERSION. Stephen W. Gray, Emory University School of Medicine.

Complete submersion of lightly anesthetized (Nembutal) rats is followed by: (1) A short period of apnea; (2) A period of hyperpnea; (3) A long period of apnea; (4) A short series of respiratory movements, regular and of about the amplitude of normal respirations, but unaccompanied by the neck and facial movements seen in phase 2.

Rats immersed in water at 1°C. reach the beginning of phase 4 in about 155 seconds, while in water at 40° the time required for the same events is only 95 seconds. The time increases again as the temperature is raised to 55° and then

decreases with higher temperatures. Similar results were obtained following administration of epinephrine, alcohol, ether anesthesia and in unanesthetized animals.

226. EFFECT OF SUPRANORMAL GRAVITATIONAL FORCE ON SWIMMING HABITS OF TADPOLES. Stephen W. Gray and Rebecca G. Webb, Emory University School of Medicine.

Developing eggs of *Bufo SP.* were centrifuged continuously for periods up to ten days at forces from one to six times gravity. Animals centrifuged for ten days at six times gravity exhibited abnormal swimming habits. They swam down and attempted to burrow through the bottom of the tubes. Two or three days after being removed from the centrifuge they acquired the usual reaction of swimming toward the surface when stimulated. No changes were found in the vestibular apparatus to account for these effects.

227. EFFECT OF SUPRANORMAL GRAVITATIONAL FORCES ON RESPIRATION AND GROWTH OF WHEAT SEEDLINGS. Aloysius I. Miller, Emory University School of Medicine. (Introduced by Stephen W. Gray.)

Wheat seeds (Sanford Winter) were subjected, in a centrifuge, to continuous gravitational force of 93.8 times normal from germination to the 24th and the 96th hour. Those centrifuged for 24 hours were allowed control conditions from the 24th to the 96th hour. Oxygen consumption was determined by an adaptation of the direct method of Warburg and growth was measured by means of a specially constructed mechanical scale.

Comparison of experimental with control data showed that those centrifuged 0-24 hours consumed significantly less total oxygen and attained a lesser total height but respired and grew at a faster rate after the 40th hour; those centrifuged 0-96 hours consumed less total oxygen and grew to a lesser height than did the controls but with similar rate of growth and O_2 uptake. They grew slower and attained a lesser height than did those centrifuged 0-24 hours. The histological picture was non-contributory. Speculation is directed toward the possible role of protoplasmic viscosity and respiratory enzyme unit bond disturbances.

228. ON SEX REVERSAL IN ADULT CLAMS, *VENUS MERCENARIA*. V. L. Loosanoff and W. S. Miller, U. S. Fish and Wildlife Service, Milford, Connecticut.

It has been shown that the gonads of many lamellibranchs contain antecedent cells of both male and female gametes, and that many of these mollusks undergo a change of sex in adult life. In some cases the change occurs during the interval between the two spawning seasons; in others, several sex changes may be experienced within a single reproductive season. In *Venus mercenaria*, as one of us has shown, the majority of the individuals pass through a protandric male phase but the adults, with few exceptions, are of separate sexes. Recent observations showed that sex reversal in adult clams is rather uncommon. This was ascertained by observations on approximately 100 clams. To determine their sex the clams were induced to spawn in the laboratory. Then their sex marks were etched on the shells

and the clams returned to the water. During the next spawning season the clams were again examined. In all instances no change of sex occurred. The only possible exception might have been one clam the shell of which was not clearly marked and, therefore, the original sex of which could not be ascertained.

229. RATE OF WATER PUMPING AND SHELL MOVEMENTS OF OYSTERS IN RELATION TO TEMPERATURE. V. L. Loosanoff, U. S. Fish and Wildlife Service, Milford, Connecticut.

Using modern methods a series of experiments was performed to determine the effects of temperature upon certain activities of oysters, shell movements and rate of pumping receiving the most attention. The range covered extended from 0.0 to 38.0°C.

Some of the oysters were moving their shells and pumping small quantities of water at temperatures less than 3.0°C. One oyster pumped at temperatures ranging between 1.3 and 1.7°C. In rare instances true feces were formed at temperatures ranging between 2.0 and 3.0°C. However, formation of pseudo feces was common at the same temperatures. Within the range from 8.0 to 16.0°C. the rate of pumping steadily increased. However, from that point to about 28.0°C. the rate of pumping showed no marked fluctuations. A further increase was again noticed within the range extending between 28.1 and 32.0°C. It is within this range that the maximum rate of pumping occurs. Between 32.1 and 34.0°C. the rate of pumping was also rapid, but beyond 34.0°C. the oysters began to show distress resulting in a marked decrease in the rate of pumping and in abnormal shell movements.

The maximum sustained rate of pumping recorded was 37,446 cc per hour, but for brief periods of 5 to 15 minutes a rate of pumping exceeding 40,000 cc per hour was recorded.

Auxiliary experiments conclusively demonstrated that the rate of pumping of oysters kept at a temperature of about 3.0 to 5.0°C. and then quickly changed to a higher temperature of about 20.0°C. began pumping at the same rate as the control oysters which were kept steadily at 20.0°C. These observations indicate that the response of the oysters to change in the environment and adjustment to such changes are extremely rapid.

230. ON FOOD REQUIREMENTS OF LARVAE OF *OSTREA VIRGINICA*. Harry C. Davis, U. S. Fish and Wildlife Service, Milford, Connecticut. (Introduced by V. L. Loosanoff.)

Our experiments have shown that the types of micro-organisms, that can be utilized as food by the larvae of *Ostrea virginica*, must be very limited. Although *Chlorella* sp. was the most satisfactory of a number of micro-organisms tried, it did not give consistently good results when fed to larvae during the early stages. However, once the larvae had reached 125 microns in size they appeared to utilize *Chlorella* sp. For example, during the summer of 1950 only a small percentage of larvae could be grown to a size of 125 μ , however, when fed *Chlorella* sp., almost all that did reach this size continued to grow and metamorphosed.

In addition to *Chlorella* sp., four species of bacteria, *Vibrio marinofulvus*, *Micrococcus maripuniceus*, *Bacillus imomarinus*, and one of the red sulfur bacteria, as well as three species of flagellates, *Chlamydomonas* sp., *Astasia klebsii*, and one of the colorless monads, were tried as food for young oyster larvae. In low concentrations, both the bacteria and the flagellates appeared to have no effect on the rate of growth nor on survival of young larvae. When fed in excess, they caused an increase in mortality. Another of the small green algae, *Stichococcus bacillaris*, was utilizable, but like *Chlorella* sp. did not give consistently good results.

Detritus from several sources did not appear to be a good food. When dissolved glucose, yeast extract or other organic media were added no increase in rate of growth was noted at low concentrations, while at higher concentrations the larvae were killed by the dense bacterial growth.

231. COMPARISON OF EFFECT OF PYRIBENZAMINE ON NORMAL AND NEOPLASTIC GRAFTS IN AMPHIBIA.¹ S. Meryl Rose, Merle Mizell and Charles F. Ellerman, University of Illinois.

Homoplastic grafts of 5 mm² pieces of skin in *Rana pipiens* remained alive an average of 22 days when hosts were kept either in tap water or in a solution of pyribenzamine containing 5×10^{-6} gm per 100 ml of tap water. Skin grafts between *Triturus viridescens* and *Rana pipiens* were destroyed by the 12th day in both a control and a pyribenzamine series. The time of survival of renal carcinomas from *Rana pipiens* adults to tails of *R. pipiens* tadpoles was several weeks and no appreciable difference in survival time was found in a control and a pyribenzamine series. The concentration of leucocytes is approximately half during the first 3 days when pyribenzamine is present. On the 5th day when the number of leucocytes had already decreased in the control hosts, the number of leucocytes present around the grafts of the experimental animals had increased to approximately the maximum number which had been present in the controls two days before. The pyribenzamine had delayed the time for maximum concentration of leucocytes two days, but had not appreciably affected graft survival time.

Normal renal tissue and a renal carcinoma from *Rana pipiens* grafted subcutaneously to *Triturus viridescens* were destroyed before 4 days when the hosts were kept in tap water. When hosts were immersed in the pyribenzamine solution 2 days before the transplantation and kept in it, the normal kidney transplants disappeared but tumor transplants lived until the end of the experiment, and judging from their appearance in histological preparations, would have lived some days longer.

It appears that pyribenzamine is useful in establishing xenoplastic tumor grafts which otherwise would be killed by immediate reaction between host and graft. The final survival time, supposedly determined by the induction of anti-bodies, is not changed by pyribenzamine.

¹ This work has been aided by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

232. METHODS OF ESTABLISHING THE LUCKÉ CARCINOMA IN *TRITURUS VIRIDESCENS*.¹
S. Meryl Rose, Florence C. Rose and Hope M. Wallingford, University of Illinois.

Renal carcinomas from *Rana pipiens* have been successfully established in forearms of *Triturus viridescens* by two methods. Establishment of a graft was verified by histological study of biopsy material. Such grafts showed some cells in mitosis and typical morphology and arrangement of cells. Grafts which had failed had been replaced by host connective tissue.

Method 1: Bits of tumor tissue 1 mm³ were grafted subcutaneously while hosts were anesthetized by MS222 (Sandoz) and chilled in cracked ice. Within a few minutes of the transplantation they were transferred to a refrigerator at 6°C. for two weeks, except for 15 minute sojourns at room temperature 2 or 3 times per day. Maintained at room temperature for 78 days after removal from the refrigerator, 8 of 14 animals gave positive biopsies on the 7th-78th days. Only 2 of 105 transplantations were successful in hosts treated identically but not chilled during the operation. Two tumors were used in the successful series, four others in the unsuccessful series.

Method 2: The newt hosts were kept in concentrations between 5×10^{-5} and 5×10^{-6} gm of pyribenzamine per cent of tap water for 2 days prior to transplantation and for 23 days thereafter, but were not cooled at any time. Positive biopsies between the 10th and 23rd days were obtained from the six experimental animals. Six controls, without benefit of pyribenzamine had destroyed their grafts before the tenth day. The same tumor was used for grafting to both experimentals and controls.

It seems likely that in our experiments the effects of heterotoxins (Lumsden, Am. J. Cancer, 1931) which act immediately after transplantation can be lessened by cooling or by treatment with antihistamine.

¹ This work has been aided by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

233. THE EFFECTS OF HYPOTONIC AND HYPERTONIC SOLUTIONS ON CILIARY MOVEMENT. Bernard J. Sullivan, Biological Laboratory, Boston College. (Introduced by John A. Frisch, S.J.)

The ciliary tracts in the buccal cavity of the frog, *Rana pipiens*, were perfused with concentrations of Ringer-Locke solution ranging from a dilution of $1/8 \times$ isotonic solution to a concentration of $4 \times$ isotonic solution. The rate of ciliary movement was estimated by measuring the time required for a cork disc, of standard size and weight, to traverse a one centimeter distance in the tract. The following observations were made: velocity of the standard object, measured at 3 minute intervals after perfusion; the time required to produce ciliary immobility; the time required to produce recovery of ciliary movement after perfusion with isotonic solution, and the minimum time of perfusion with hypotonic and hypertonic solutions which resulted in irreversible damage to the cilia.

It was found that hypotonic solutions are more detrimental to ciliary activity than hypertonic solutions. Ciliary immobility and irreversible damage are produced more rapidly with hypertonic than with hypotonic solutions. Hypertonic

solutions produce a gradual but constant decline in the rate of ciliary activity while hypotonic solutions produce an initial increase and is followed by a more gradual decline in the rate of ciliary movement.

234. RESPIRATION STUDIES ON SMALL ARCTIC MAMMALS. Bernard J. Sullivan, Biological Laboratory, Boston College. (Introduced by John A. Frisch, S.J.)

The respiration studies were carried at the Arctic Research Laboratory, Point Barrow, Alaska, and were sponsored by the Arctic Institute of North America and the Office of Naval Research.

A constant pressure type of respirometer was employed, consisting of a respiration chamber and compensating chamber connected by a manometer, two calibrated burets from which measured volumes of air were introduced into the respiration chamber, and a constant temperature water bath in which the chambers were immersed. A small fan operated magnetically by means of a motor placed outside of the chamber, agitated the air in the respiration chamber and insured continuous contact of the air with the CO₂ absorbing agent.

Studies were carried out on the Arctic ground squirrel (*Citellus parryi*), the Alaska Least weasel (*Mustela rixosa eskimo*), and the lemming (*Lemmus alascensis* Merriam). Oxygen consumption was measured at temperatures from 5°C. to 25°C., at five degree intervals. Preliminary results indicate an average rate of oxygen consumption of 0.64 in the squirrel, 7.36 in the weasel, and 3.96 in the lemming, expressed in ml O₂/gm of body wt./hour.

When the rate of oxygen consumption was plotted against temperature a straight line relationship was obtained with values for the lemming and weasel, with a high rate of oxygen consumption at 5°C. and a gradual decline as the temperature was increased to 25°C.

The squirrel consumed the greatest amount of oxygen at 5°C. The curve declined at 10°C., leveled off at 15-20°C., and increased slightly at 25°C.

Genetics

235. X-RAY INDUCED SOMATIC CROSSING OVER IN *DROSOPHILA MELANOGASTER*. George Lefevre, Jr., University of Utah.

Eye color mosaics were produced by irradiating larval stages of *Drosophila* with 1300r at the rate of 59r/minute 48 hours after egg laying. The mosaic incidence in heterozygous white females was 0.65 per eye. In similar females, but also bearing a double inversion in one X-chromosome, the mosaic incidence was 0.06 per eye. In males an incidence of less than 0.02 white mosaics per eye was found. In males, mosaics result mainly from somatic mutation; in females deletion, chromosome loss, translocation, abnormal disjunction, and somatic crossing over also may produce mosaics. The presence of inversions should not alter the effectiveness of any of the mosaic-producing mechanisms, except the last. Thus, in normal heterozygous females the great majority of mosaics result from somatic crossing over, or possibly from non-homologous segmental interchange.

The distribution of induced somatic cross-overs was studied by comparing the mosaic incidence in heterozygous white (locus 1.5), heterozygous garnet (44) and heterozygous carnation (62.5) females. The results indicate that an appreciable amount of somatic crossing over must take place in the heterochromatic portion of the X-chromosome; but it is not equally distributed throughout the X-chromosome, being greater in the right than in the left end. A similar distortion of the normal pattern of crossing over is found when various agents are used in order to induce excessive germinal crossing over.

236. MORTALITY AND THIOUREA RESISTANCE, 44 GENERATIONS OF *DROSOPHILA MELANOGASTER*.¹ Morris H. Harnly and E. D. Goldsmith, Washington Square College of Arts and Science and Department of Histology, College of Dentistry, New York University.

Pair matings of the wild Woodbury stock of *D. melanogaster* were carried at 25°C. on 9 mg percent thiourea in half pint bottles for 44 generations. The mean yield rose slowly from 22 to 150 imagos. It is evident that this stock constitutes a population composed almost entirely of genotypes highly susceptible to thiourea and that a concentration of 9 mg percent is lethal to nearly the entire population. Long term selection with random matings produces a population much more resistant to the compound. Since the increase in resistance is gradual and has not ceased by the 44th generation a number of loci must be involved. Through the first 26 generations thiourea was far more toxic to the females than to the males. This difference decreased slowly and the sex ratio was normal from the 30th to the 44th generation. Evidently there are sex-linked recessive alleles as well as autosomal alleles facilitating the metabolism of thiourea. Tests of this thiourea selected line on two closely related compounds demonstrate that for one, thiouracil, thiourea selection gives no protection and for the other, phenylthiourea, only partial protection. A parallel experiment using 7.5 mg percent thiourea indicates that this lower intensity is much more effective in selecting for survival. These experiments are being continued and also extended to other related and unrelated compounds.

¹ Aided by a grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

Histology

237. THE ANATOMY, HISTOLOGY, AND CYTOLOGY OF THE THYROID GLAND OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*).¹ Florence G. Buch and Harry A. Charipper, Department of Biology, New York University, Washington Square.

Two oval shaped lobes of the thyroid gland of the golden hamster are connected by a thin isthmus and lie on the ventral surface of the trachea. They cover the first two tracheal cartilages and extend laterad and partly dorsad. The blood supply is extensive. The very small size of the gland as determined by torsion balance weights (weight range from 3 mg in a 3 month old male to 15 mg in an 8 month old female) may be indicative of the general metabolism of the hamster. The follicular epithelial cells vary in size from low to high columnar. The nuclei

are generally large and spherical and may be either centrally or basally located. The intracellular cytoplasm is granular with occasional vacuoles surrounding the nuclei. The follicles at the periphery are larger than the more central ones. Vacuoles often accumulate at the periphery of the intrafollicular colloid. The Golgi material always caps the apical side of the nucleus. Rod-shaped and granular mitochondria and other granules are scattered throughout the cytoplasm. These cytoplasmic inclusions appear to bear a possible relation to functional activity of the thyroid gland in the golden hamster. This study should serve as the basis for future investigations on the thyroid-gonad-hypophysis relationship to the complex rather evasive process of aging.

¹ Rockefeller Institute for Medical Research strain, ranging in age from 3 months to 15 months.

Parasitology

238. PARASITES OF THE RANIDAE (AMPHIBIA). XXI. A. C. Walton, Knox College.

The annotated catalog of the parasites of the genus *Rana* continues the list of hosts and their parasites as follows: 89a. *Rana tigerina* (Burma, Ceylon, India) — *Camallanus baylisi*, *Camallanides prashidi*, *Gendria ranarum*, and *Oxysomatium macintoshii* (Nematoda); *Diplodiscus amphichrus*, *D. amphi. magnum*, larval *Encyclomet. a colubrimurorum*, *Ganeo glottoides madrasensis*, *G. korkei*, *G. tigrinum*, *Halipegus longispina*, *H. mehransis*, *H. mehr. minutum*, *Mehraorchis ranarum*, *Mesocoelium sociale*, *Phyllodistomum shandrai*, *Pleurogenes gastroporus*, *P. gastro. equalis*, *P. medianequalis*, *P. prayagi*, *Prosotocus confusus indiana*, *P. indicus*, and *Tremiorchis ranarum* (Trematoda); larval *Diphylobothrium erinacei*, and *Proteocephalus tigrinus* (Cestoda); larval *Centrorhynchus aluconis* (Acanthocephala); *Anaplasma* sp? de Mello ('17), *Balantidium ?duodeni*, *B. ?elongatum*, *B. gracile*, *B. helenae*, *B. sp?* Dobell ('10), *Cepedea ophis*, *C. seychellensis angusta*, *C. virgula*, *Dactylosoma ranarum*, *D. splendens*, *Entamoeba ranarum*, *Eutrichomastix ?batrachorum*, *E. sp?* Walton ('41), *Haemogarina berestnieffi* (? = *H. sp?* Berestnev, '02), *H. sp?* Dobell ('10), *H. sp?* Franca ('17), *H. sp?* Houdemer ('27), *Hexamita* sp? Walton ('41), *Lankesterella minima* (= *L. monila*), *Nycototherus ?cordiformis*, *N. macropharyngeus*, *N. papillatus*, *Opalina annandali*, *O. coracoidea*, *O. coralahorensis*, *O. ranarum*, *O. sp?* Dobell ('10), *Protoopalina filiformis*, *Trichomonas batrachorum*, *T. sp?* Dobell ('10), *Trypanosoma borrelli*, *T. hendersoni*, *T. inopinatum* (also in tadpoles), *T. rotatorium*, *T. sp?* Dobell ('10), *T. sp?* Laveran & Mesnil ('12), and *Zschokkella prashadi* Ray ('33) (nomen nudum ?) (Protozoa); *Glossophonia* sp? Budde-Lund ('74), and *Hemiclepsis viridis* (Annelida); and larval *Kiricephalus pattoni* (Pentastomida).

239. PARASITES OF THE RANDAE (AMPHIBIA). XXII. A. C. Walton, Knox College,

89b. *Rana tigerina* (Fr. Indo-China) — *Icosiella neglecta*, and *Microfilaria* sp? Mathis & Leger ('11) (Nematoda); *Glypthelmins ?linguatula*, and *Prosotocus confusus indiana* (Trematoda); plerocercoids of *Diphylobothrium erinacei*

(Cestoda); *Acanthocephalus lucidus*, *A. tigrinae*, and larval *Echinorhynchus* sp? Houdemer ('27) (Acanthocephala); and *Haemogregarina scheini* (? = *H.* sp? Schein, '11), *H.* sp? Mathis & Leger ('11), *Trypanosoma chattoni*, and *T.* sp? Mathis & Leger ('11) (Protozoa). 89c. *Rana tigrina* (Java) — *Filaria* sp? Baylis ('33) (Nematoda); larval *Centrorhynchus* sp? Baylis ('33) (Acanthocephala); and larval *Kiricephalus pattoni* (Pentastomida). 89d. *Rana tigrina* (Zool. Gard., England) — *Rhabdias bufonis* (Nematoda), and *Haemogregarina* sp? Scott ('28), *H.* sp? Wenyon ('31), *H.* sp? Wenyon ('34), *Hexamita* sp? Scott ('26), *Lankesterella* sp? Scott ('26), *L.* sp? Scott ('28), *L.* sp? Wenyon ('31), *L.* sp? Wenyon ('32), *Trypanosoma* sp? Scott ('28), and *T.* sp? Wenyon ('28) (Protozoa).

240. PARASITES OF THE POLYPEDATIDAE (AMPHIBIA). A. C. Walton, Knox College.

1. *Hylambates rufus* (Africa) — *Balantidium* sp? Metcalf ('23), *Nycototherus* sp? Metcalf ('23), and *Opalina camerunensis* (Protozoa). 2. *Hyperolius concolor* (Africa) — *Haplometroides rappiae* (Trematoda); and *Nematotaenia janickii* (Cestoda). 3. *H. fulvovittata* (Africa) — *Nematotaenia janickii* (Cestoda). 4. *H. marmoratus* (Africa) — *Cepedea madagascariensis*, *Haemogregarine pestanae*, *Protoopalina intestinalis*, *Trypanosoma* sp? Dutton, et al. ('07), and *T.* sp? Laveran & Mesnil ('12) (Protozoa). 5. *H.* sp? (Africa) — *Haemogregarina hyperolii* (Protozoa). 6. *Leptopelis aubryi* (Africa) — *Oxysomatium hylambates* (Nematoda). 7. *L.* sp? (Africa) — *Trypanosoma rotatorium* (Protozoa). 8. *Mantella baroni* (Madagascar) — *Opalina mantellae* (Protozoa). 9. *Mantidactylus luteus* (Madagascar) — *Schöngastia madagascariensis* (Acarina). 10. *Megalixalus madagascariensis* (Madagascar) — *Cepedea madagascariensis* (Protozoa). 11. *M. seychellensis* (Seychelles) — *Cepedea seychellensis* (Protozoa). 12. *Polypedates buergeri* (Japan) — *Pharyngodon polypedatis*, and *Rhabdias polypedates* (Nematoda); *Cryptotropa kuretanii*, *Gorgoderina kajika*, *Mesocoelium japonicum*, and *Pleurogenes lobatus* (Trematoda); *Acanthocephalus lucidus* (Acanthocephala); *Cepedea buergeri* (Protozoa); and *Oligobdella orientalis* (Hirudinea). 13. *P. eques* (Ceylon) — *Mesocoelium marrsi* (Trematoda); and *Opalina zeylonica* (Protozoa). 14. *P. leucomystax* (Ceylon, India, Indo-China, Java, Sumatra) — *Cepedea segmentata*, *C. virgula*, *Trypanosoma* sp? Laveran & Mesnil ('12), and *T.* sp? Mathis & Leger ('11) (Protozoa). 15. *P. leuco. megacephalus* (China) — (in tadpoles and adults) larval *Pharyngostomum cordatum* (Trematoda). 16. *P. maculatus* (Ceylon, India) — *Mesocoelium burti* (Trematoda); and *Cepedea longa*, *C. thiagi*, *C. virgula*, and *Nycototherus papillatus* (Protozoa). 17. *P. rein. wardtii* (Borneo) — *Protoopalina borneonensis* (Protozoa). 18. *P. rhodoscelis* (Madagascar) — *Cepedea lemuria* (Protozoa). 19. *P. schlegeli* (Japan) — *Rhabdias rhacophori* (Nematoda); and *Cepedea ?multiformis* (Protozoa). 20. *P. schl. arborea* (Japan) — *Oswaldocruzia bialata* (Nematoda); *Polystoma rhacophori* (Trematoda); and *Baerietta japonica* (Cestoda).

Protozoology

241. COMPARATIVE BEHAVIOR IN THE MATING AND CONJUGATION OF OPPOSITE MATING TYPES IN DIFFERENT CLONES OF *PARAMECIUM BURSARIA*.¹ Ralph Wichterman, Temple University.

When opposite mating types of different clones of *Paramecium bursaria* are crossed and the mating reactions, isolated conjugants, exconjugant stages and their progeny compared, certain differences are noted.

The procedure consisted of mixing and mating the opposite types of different clones, isolation of large numbers of conjugants as soon as possible after mating and daily observations of the conjugants, exconjugants and their progeny for at least two weeks after each experiment. Isolated vegetative specimens were used as controls in each experiment and the cultural conditions for all clones were identical.

Opposite mating types as collected from nature and cultivated in the laboratory for only one year showed, when mixed, a more rapid clumping reaction and the formation of larger clumps than considerably older clones. In such matings, the younger clones produced conjugants which remained joined together in the sexual process for a longer period than older clones maintained in the laboratory for 10 years. While this appears to be due to clonal age as cultivated in the laboratory, a racial or genetic factor may be involved to account for duration of time conjugants remain joined together.

Also greater variation in regard to length of time conjugants remained joined, appeared in the younger clones. In one such instance, a pair of isolated conjugants remained joined for five days yet the exconjugants produced flourishing progeny.

In all cases, the most critical stage for survival in conjugation appears to be at the end of the conjugation period or at the beginning of the exconjugant stages. It is significant that the vegetative control specimens allowed to divide by fission alone more frequently produced successful progeny than the exconjugants.

¹ Aided by a grant from the Committee on Research and Publication, Temple University.

AUTHOR INDEX

Showing abstract numbers and pages for each author

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Adams, A. E.	176	594
Adamson, D. M.	15 16 134	516 572
Adamstone, F. B.	60	538
Akers, R. P.	76	544
Alfert, M.	42	530
Allen, E.	143 205	576 609
Allison, J.	168	589
Altland, P. D.	143 204 205	576 608 609
Anderson, N. G.	39	528
Angelone, L.	222	617
Angerer, C. A.	221 222	616 617
Antonchak, N. W.	96	554
Armen, G. K.	179	595
Aronson, L. R.	87	550
Aull, J. C.	215	614
Baker, A. S.	26	522
Ball, S. C.	149	580
Barnes, T. C.	108	560
Barnnett, R. J.	34 35	525 526
Barth, L. G.	162 163	587
Barth, L. J.	162 163	587
Beams, H. W.	7 65	512 540
Bennett, T.	183	597
Berg, G. G.	21	519
Berg, W. E.	56	536
Bern, H. A.	32 122	524 567
Beutner, R.	108	560
Bingham, H.	57	537
Bishop, D. W.	136 137	573 574
Bodine, J. H.	55	536
Boss, W. R.	184	598
Brown, F. A.	196 198	604 605
Brown, M. M.	85 86 171	549 591
Bruner, J. A.	170	590
Buch, F. G.	237	624
Buck, J. B.	8 9 207	512 513 609
Bullock, J. A.	50	533
Bullock, T. H.	201	607
Calhoon, T. B.	221	616

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Calhoun, J. B.	83	538
Carpenter, C. C.	130	571
Charipper, H. A.	194 237	603 624
Cheney, R. H.	5	511
Clark, E.	87	550
Clark, H.	180	596
Collias, N. E.	92 93	552 553
Collin, J.	200	606
Comer, R.	57	537
Cornman, I.	54	535
Costello, D. P.	146	578
Coulombre, A.	84	548
Craig, M.	176	594
Crary, D. D.	160	586
Creaser, C. W.	148	579
Crowell, S.	109	560
Dalton, H. C.	19	518
Davis, D. E.	98	555
Davis, H. C.	230	620
Denny, D.	167	589
Dent, J. N.	27	522
Dougherty, E. C.	11	514
Dougherty, R. W.	49	533
Easton, T. W.	12	515
Elias, H.	128	570
Ellerman, C. F.	231	621
Emlen, J. T., Jr.	90 131	551 571
Etkin, W.	67	541
Evans, H. J.	183	597
Evans, T. C.	65	540
Everett, J. W.	182	596
Falk, D.	177	594
Farmelant, M. H.	97	555
Feder, N.	102	557
Feightner, L. E.	141	575
Ferguson, M. S.	80	546
Ferguson, R.	38	528
Fingerman, M.	196	604
Finley, H. E.	46 47 64	532 540
Flax, M. H.	40 62	529 539
Ford, D. H.	173	592
Forster, R. P.	3 106	510 559
Freedman, H. H.	186	599
Freeman, J. A.	100	556
Fuller, J. L.	81	547
Fulton, G. P.	76 77	544 545
Garrett, F. A.	29	523

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Geheuo, P. M.	75	544
Gennaro, J. F., Jr.	37	527
Gier, H. T.	111	561
Giese, A. C.	209 210 211 212	610 611 612
Gilbert, P. W.	17	517
Goldsmith, E. D.	117 236	564 624
Goldstein, M.	71	543
Gonzales, F.	74	543
Goodman, M.	10 202	514 607
Gordon, A. S.	186 187 188	599 600
Gray, I. E.	125	568
Gray, S. W.	225 226	618 619
Green, E. L.	126	568
Green, M. C.	124	568
Greep, R. O.	34 35	525 526
Griffin, D. R.	63	540
Grun, J. A.	172	591
Guyselman, J. B.	197	604
Gwilliam, G. F.	213	613
Haack, M. E.	178	595
Haig, C.	203	608
Hall, E. K.	79	546
Hamilton, H. L.	165	588
Handler, A. H.	77	545
Hansborough, L. A.	153 167	582 589
Hard, W. L.	144	577
Harnly, M. H.	117 236	564 624
Hartung, E. W.	41	530
Hawkins, R. K.	144	577
Heilbrunn, L. V.	53	535
Henley, C.	146	578
Hertzler, E. C.	129	570
Hiestand, W. A.	216 217 218	614 615
Higginbotham, A. C.	110	565
Highman, B.	204	608
Hill, R.	118	565
Himes, M. H.	40 62	529 539
Hines, M. N.	196	604
Hisaoka, K.	115	563
Hisaw, F. L.	97	555
Hitchcock, H. B.	132	571
Holtzer, H.	219	615
Hopper, A. F.	95	554
Howell, C. D.	121	566
Howell, C. H.	96	554
Huddleston, Sister M. S.	140	575
Hungate, R. E.	49	533

AUTHOR INDEX

631

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Hunter, F. R.	50	533
Huston, M. J.	4	510
Ingles, L. G.	192	602
Ito, S.	72	543
Jakowska, S.	127	569
Jewell, H. A.	194	603
Kahn, R. H.	122	567
Kaltenbach, J. C.	36 118	526 565
Karczmar, A. G.	21	519
Kassel, R.	135	573
Keeler, C. E.	61	539
Keister, M. L.	8 9	512 513
Kelly, J. W.	53	535
Kemp, N. E.	18	517
Kendeigh, S. C.	89	551
Khan, M.	153	582
Kiao-Hung Lu	55	536
Kiebel, G.	214	613
Kile, J. C., Jr.	101	557
Kinard, F. W.	215	614
King, R. L.	66	541
Kitchell, R. L.	185	598
Klenner, J. J.	37	527
Klopp, C. T.	33	525
Knobil, E.	30 31	523 524
Kollros, J. J.	118	565
Kopac, M. J.	44 135 145	531 573 578
Kraemer, D.	69	542
Krueger, K. K.	51	534
Kutsky, P. B.	56	536
Landau, J.	138 139	574
Latimer, H. B.	157 158 159	584 585
Leathem, J. H.	28	522
Lefevre, G., Jr.	235	623
Leonard, S. L.	31	524
Leshner, S. W.	52	534
Lessler, M. A.	44 145	531 578
Loosanoff, V. L.	147 228 229	579 619 620
Ludwig, D.	208	610
Lutz, B. R.	76 77	544
Luyet, B. J.	74 75 78	543 544 545
McCord, W. M.	215	614
Madison, M.	57	537
Makinodan, T.	202	607
Markee, J. E.	182	596
Markert, C. L.	119	565
Markoff, H.	190	601

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Marsh, G.	7	512
Marsland, D.	138 139	574 575
Martin, A. W.	4	510
Martin, C.	177	594
Massonneau, J.	199	606
May, R. M.	199 200	606
Mayer, W. V.	193	602
Megel, H.	187	599
Menz, L.	78	545
Merklin, R. J.	28	522
Metz, C. W.	43	530
Michalski, J. V.	223	617
Miller, A. I.	227	619
Miller, W. S.	228	619
Mitchell, M. J.	220	616
Mizell, M.	231	621
Moment, G. B.	113	562
Moog, F.	114	563
Moore, J. A.	2	509
Morbeck, F. E.	151	581
Morris, D. M.	94	553
Neher, G. M.	99	556
Nelsen, O. E.	155	583
Nelson, E.	51	534
Nicholas, P. A.	47	532
Nigrelli, R. F.	127	569
Norton, S.	10	514
Osborn, C. M.	178 184	595 598
Overton, J.	166	589
Palm, J.	59	537
Passoneau, J. V.	104	558
Patt, D. I.	77	545
Pearse, A. S.	1	509
Pepernik, V.	118	565
Peterson, R. R.	85 86 171	549 591
Pottinger, M. A.	14	515
Pritchard, A. W.	212	612
Ramey, R.	195	603
Rauch, H.	224	618
Rayner, B.	85 86 171	549 591
Reynolds, J. P.	43	530
Risley, P. L.	181	596
Roberts, H. S., Jr.	39	528
Rogan, J. F.	164	587
Rogers, D.	210	611
Romanoff, A. L.	154	583
Romanucci, D.	112	562

AUTHOR INDEX

633

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Ronkin, E.	48	533
Ronkin, R. R.	48	533
Roofe, P. G.	57	537
Rosa, C. G.	156	584
Rose, F. C.	22 232	519 622
Rose, S. M.	22 141 231 232	519 575 621 622
Roth, A. B.	141	575
Rulon, O.	116 152	564 582
Runner, M. N.	59	537
Russell, L. B.	25	521
Russell, W. L.	25	521
Sabella, J.	122	567
Salhanick, H. A.	97	555
Sanborn, R. C.	103	558
Sandeen, M. I.	198	605
Sandow, A.	214	613
Saunders, J. W., Jr.	24 150 151	520 581
Sawin, P. B.	158 159 160	584 585 586
Sawyer, C. H.	182	596
Schipper, A. L.	13 14	515
Schneiderman, H. A.	102	557
Shrier, J.	165	588
Schwartz, C.	38	528
Scott, E. B.	38	528
Scott, J. P.	81	547
Scott, J. W.	91	552
Seaman, G. R.	107	559
Sedar, A. W.	45	531
Seigel, G. B.	142	576
Shaw, E. S.	88	550
Shieman, R. D.	73	543
Siers, M. R.	110	561
Singer, M.	20	518
Smith, C.	190 191	601
Smith, J.	70	542
Smith, T. C.	97	555
Soderwall, A. L.	179 195	595 603
Stahlecker, H. A., Jr.	175	593
Stein, K. F.	177	594
Stemler, F. W.	217 218	614 615
Stephens, G. C.	198	605
Stevens, D. F.	77	545
Strand, F. L.	188	600
Strecke, R. L.	131	571
Stroud, C. P.	133	572
Sullivan, B. J.	233 234	522 623
Taggart, J. V.	106	559

<i>Author</i>	<i>Abstract No.</i>	<i>Page</i>
Tahmisian, T. N.	15 16 134	516 572
Talmage, R. V.	29	523
Tedford, M. D.	181	596
Telfer, W. H.	105	559
Thornton, C. S.	69	542
Trinkaus, J. P.	161	586
Van Brocklin, G.	195	603
Van der Kloot, W.	6	511
Vogel, H. H., Jr.	82	547
von Bertalanffy, L.	123	567
Waldron, M. J.	191	601
Wallingford, H. M.	232	622
Walton, A. C.	238 239 240	625 626
Wassermann, F.	68	542
Watkins, D. H.	222	617
Webb, H. M.	196	604
Webb, R. G.	226	619
Weiss, P.	150	581
Whiting, A. R.	206	609
Wichterman, R.	241	627
Wiebers, J. E.	217 218	614 615
Wilde, C. E., Jr.	23	520
Williams, C. M.	6 102 103 104 105	511 557 558 559
Wilson, D. F.	45	531
Wilson, F. H.	58	537
Witschi, E.	168 169	589 590
Wolfe, H. R.	10 202	514 607
Wolfson, A.	174 175 220	592 593 616
Worley, L. G.	142	576
Young, W. C.	85 86 171 172 173	549 591 592
Yushok, W. D.	154	583
Zarrow, I. G.	189	600
Zarrow, M. X.	99 189	556 600
Zwilling, E.	120	566